TP53BP2 decreases cell proliferation and induces autophagy in neuroblastoma cell lines

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Abstract. Tumor protein p53-binding protein 2 (TP53BP2), a member of the apoptosis-stimulating protein of p53 (ASPP) family, has previously been reported to be associated with tumor development. However, to the best of our knowledge, the role of TP53BP2 in neuroblastoma has not been elucidated. The aim of the present study was to investigate the function of TP53BP2 in the proliferation and autophagy of neuroblastoma. An expression vector that expresses TP53BP2-specific short hairpin RNA (shTP53BP2) was used for the experimental group and green fluorescent protein short hairpin RNA was used as a control. Cell proliferation was measured using MTT assays, self-renewal was evaluated using soft agar assays, light chain 3 (LC3) II expression level was examined by western blot and immunofluorescence analysis, and the autophagy-related 3 homolog (ATG3), autophagy-related 5 homolog (ATG5) and autophagy-related 9 homolog (ATG7) expression levels were examined using the reverse transcription-quantitative polymerase chain reaction (RT-qPCR). A genomics analysis revealed that TP53BP2 expression was associated with the survival of patients with neuroblastoma. Western blot and RT-qPCR assays indicated that TP53BP2 could be implicated in neuroblastoma, as the proliferative ability of the experimental group decreased compared with that of the control group (P<0.001) and the expression levels of genes associated with autophagy, including LC3 II, ATG3, ATG5 and ATG7, increased in the experimental group. In conclusion, an increased expression of TP53BP2 in patients with neuroblastoma may be associated with poor survival and shTP53BP2 may decrease the proliferative abilities of neuroblastoma cells, including BE(2)C and SK-N-DZ cell lines. In addition, the LC3 II, ATG3, ATG5 and ATG7 expression levels increased and were associated with increased rates of autophagy following upregulation of TP53BP2.

Introduction

Neuroblastomas are common pediatric extracranial tumors of neural crest origin that account for 10% of cancer cases in children and ~15% of cancer-associated mortalities in children (1-6). Clinical features of neuroblastoma include heterogeneity, high malignancy and metastasis (7,8). Common therapeutic methods include surgery, radiotherapy and chemotherapy, and novel methods include immunotherapy and differentiation therapy; however, the treatment of neuroblastoma remains unsatisfactory and the prognosis is poor (9). Therefore, an increasing number of studies have aimed to identify feasible biotherapies and drug targets (10).

TP53BP2 is a member of the apoptosis-stimulating protein of p53 (ASPP) family, which can regulate p53-dependent apoptosis (11). A number of studies have indicated that TP53BP2 is overexpressed in various tumor types, and is a critical factor in tumorigenesis and development (12,13). TP53BP2 inhibits squamous cell carcinoma by regulating p63 (14). In breast cancer, overexpression of TP53BP2 is often associated with a poor prognosis, and TP53BP2 can interact with microRNA-548d-3p to regulate proliferation and apoptosis (15). In gastric cancer, the expression of TP53BP2 is associated with tumor stage (16). Survival data from R2 genomic analyses in the present study indicate that TP53BP2 may be associated with the prognosis of patients with neuroblastoma; however, to the best of our knowledge, the role and molecular mechanisms of TP53BP2 in neuroblastoma have not been reported. Therefore, the aim of the present study was to investigate the mechanism of TP53BP2 in neuroblastoma and provide a theoretical basis for clinical treatment.

Autophagy is an evolutionally conserved mechanism that can degrade organelles, proteins, macromolecules and ribosomes via lysosomes, which is critical for the maintenance of intracellular stability and stress responses (17). There are four types of autophagy, including macroautophagy (also...
termed autophagy), selective autophagy, microautophagy and chapter-one-mediated autophagy (18-20). Autophagy consists of several key steps, including initiation, nucleation, expansion and maturation of autophagosomes. A number of autophagy-related (ATG) genes participate in autophagy (21-23). Previously, autophagy has been reported to be associated with pathological and disease processes, including infectious diseases, autoimmune diseases, myopathy, neurodegenerative diseases and cancer (21,24,25). The present study demonstrated that TP53BP2 can regulate proliferation and autophagy of neuroblastoma cells. Knockdown of TP53BP2 inhibited cell proliferation and increased the expression level of LC3 II (also termed LC3B). LC3 I is the precursor of LC3 II; LC3 I is activated when autophagy occurs and induces the production of LC3 II, which promotes autophagy (26). In summary, the results of the present study revealed that TP53BP2 may be used as a prognostic marker for neuroblastoma and may regulate the proliferation of neuroblastoma cells.

Materials and methods

Cell culture. The human neuroblastoma cell lines SK-N-AS, BE(2)C, SK-N-DZ, SK-N-F1 and SHEP1 were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). The BE(2)C cells were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's nutrient mixture F12 (DMEM/F12; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin (P/S). SK-N-AS, SK-N-DZ, SK-N-F1 and SHEP1 cells were cultured in DMEM (Thermo Fisher Scientific, Inc.) with 10% FBS and 1% P/S. The 293FT cell line (ATCC) was used for cell transfection, according to the manufacturer's protocol. 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) was used for cell transfection the 293FT cell line with the packaging plasmids pLP1, pLP2 and pLP/VSVG (all from Invitrogen; Thermo Fisher Scientific, Inc.) and the shRNA plasmids. Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) was used for all transfections, according to the manufacturer's protocol. After 48 h, virus-containing media were harvested. BE(2)C and SK-N-DZ cells were plated at a density of 5x10⁴ cells in 100 mm plates and cultured for 24 h. Subsequently, the virus-containing media were mixed with 4 µg/ml Polybrene (Invitrogen; Thermo Fisher Scientific, Inc.) and used for cell infection. At 24 h after infection, the medium was removed and cells were cultured with 2 mg/ml puromycin for 2 days. The cells were then selected for subsequent experiments.

Cell proliferation assay. MTT assays were used to investigate cell proliferation. Briefly, ~1,000 cells were seeded in 96-well plates with 200 µl DMEM/F12. After 24 h of culture, 20 µl MTT was added to each well and the cells were incubated for 4 h. Dimethylsulfoxide was added to the wells to dissolve the purple formazan crystals. After 10 min on a shaking table, the absorbance was determined at 560 nm using a microplate reader daily for 7 days. All experiments were performed independently in triplicate.

5-Bromo-2-deoxyuridine (BrdU) staining assay. For BrdU immunofluorescence staining, BE(2)C and SK-N-DZ cells were cultured in 24-well plates and incubated with 10 µg/ml BrdU for 45 min at 37°C. The cells were then washed with PBS three times and fixed for 20 min with 4% paraformaldehyde (PFA) at room temperature. Subsequently, the cells were exposed to 0.3% Triton X-100 for 5 min, treated with 1 mol/l HCl and blocked for 1 h with 10% goat serum (Thermo Fisher Scientific, Inc.) diluted with PBS at room temperature. The cells were then incubated with a monoclonal rat primary antibody against BrdU (1:200; catalog no. ab6326; Abcam, Cambridge, UK) for 1 h at room temperature and with Alexa Fluor® 594 goat anti-rat immunoglobulin G (IgG) secondary antibody (1:400; catalog no. A-21211; Invitrogen; Thermo Fisher Scientific, Inc.) for 1 h at room temperature. DAPI (300 nM) was used for nuclear staining for 15 min at room temperature. A Nikon 80i fluorescence microscope (Nikon Corporation, Tokyo, Japan) was used to observe the number of stained cells (magnification, x200). Images of ten randomly selected microscopic fields were captured.

Lentiviral infection. TP53BP2 short hairpin (shRNA) and green fluorescent protein (GFP) shRNA were purchased from BGI (Shanghai, China). The sequences of TP53BP2 shRNA (shTP53BP2) were as follows: shTP53BP2-1# forward, 5'-CACCAGCAAGATGCCAAGCTACACAGCGAAGTTGATGCTTGCAATTCTGC-3' and reverse, 5'-AAAGAGCAGATGCCAAGCTACACATTCTGTGTGACCTGTGCGAT TCTGC-3'; and shTP53BP2-2# forward, 5'-CACCAGCTGC AGTATGCTCCTATATCCGCAGATGATGACACTGCAGC-3' and reverse, 5'-AAAGAGCAGATCCAGTAGGTCCCTA TATCTTCGGAATGAGTACACTGCAGC-3'. The PLKO.1 vector (Thermo Fisher Scientific, Inc.) was digested using AgeI and BamHI, and the annealed human TP53BP2 shRNA was inserted into the PLKO.1 vector using T4 ligase. Subsequently, the vector was transformed and monoclonal clones were selected for sequencing. The plasmids were extracted using a plasmid extraction kit (Tiangen Biotech Co., Ltd., Beijing, China). Lentiviruses were generated by co-transfecting the 293FT cell line with the packaging plasmids pLP1, pLP2 and pLP/VSVG (all from Invitrogen; Thermo Fisher Scientific, Inc.) and the shRNA plasmids. Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) was used for all transfections, according to the manufacturer's protocol.
ATG5 and ATG7 were determined using the SYBR Green PCR Master mix (Takara Bio, Inc.) using qPCR. The qPCRs were performed in triplicate using the OneStep plus7500 RT-PCR system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The amplification conditions were as follows: 95°C for 10 min, 95°C for 15 sec, 60°C for 30 sec and 35 cycles of 30 sec at 72°C. The following primer sequences were used: GAPDH forward, 5'-ACG GAT TTG GTC GTA TTG GG-3' and reverse, 5'-TCC TGG AAG ATG GTG ATG GG-3'; TP53BP2 forward, 5'-AGT CAG TTC CTT GTG GAG CC-3' and reverse, 5' -CCG CAG AAA CAC CTG TGA AC-3'; ATG3 forward, 5'-TTG GCT ATG ATG AGC AAC GG-3' and reverse, 5' -CCC ATC CAG AGT TGC TTG TGA-3'; ATG5 forward, 5'-TCA GCT CTT CCT TGG AAC ATC A-3' and reverse, 5' -CCC ATC CAG AGT TGC TTG TGA-3'; and ATG7 forward, 5' -TTT GCT TCC GTG ACC GTA CC-3' and reverse, 5' -CTT TTC TCC CAT CAC ACT GCT TTA-3'. The data were analysed using the 2^{-ΔΔCq} method (27). GAPDH was used as the control.

Western blot assay. SK-N-AS, BE(2)C, SK-N-DZ, SK-N-F1 and SHEP1 cells were digested with trypsin and washed twice with PBS. Cells were harvested and suspended in 1% radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Haimen, China). Protein concentrations were measured using a Bicinchoninic Acid protein assay kit (Beyotime Institute of Biotechnology). Total protein (50 µg) was separated using SDS-PAGE (30% gel) and then the proteins were transferred onto polyvinylidene difluoride membranes. Following blocking for 2 h with 5% goat serum (diluted with PBS) at room temperature, the membranes were incubated overnight at 4°C with the following primary antibodies: Anti-TP53BP2 (1:1,000, catalog no. ab181377; Abcam), anti-LC3 II (1:1,000; catalog no. ab48394; Abcam) and anti-tubulin (1:5,000; catalog no. ab7291; Abcam). The membranes were than incubated at room temperature for 2 h with horseradish peroxidase-conjugated anti-mouse IgG secondary antibody (1:1,000; catalog no. ab181377; Abcam), anti-LC3 II (1:1,000; catalog no. ab48394; Abcam) and anti-tubulin (1:5,000; catalog no. ab7291; Abcam). The membranes were then incubated at room temperature for 2 h with horseradish peroxidase-conjugated rabbit anti-goat IgG secondary antibody (1:10,000; catalog no. 04-18-06) or horseradish peroxidase-conjugated rabbit anti-goat IgG secondary antibody (1:10,000; catalog no. 04-18-06) or horseradish peroxidase-conjugated rabbit anti-goat IgG secondary antibody (1:10,000; catalog no. 14-13-06; both from KPL, Inc., Gaithersburg, MD, USA). The membranes were washed three times with washing buffer, and the Western blot signals were visualized using BeyoECL Plus reagent (Beyotime Institute of Biotechnology). Western blot data were quantified with Gel-Pro Analyzer 4 software (Media Cybernetics, Inc., Rockville, MD, USA).

Immunofluorescence assay. The expression of LC3 II was detected using immunofluorescence. In total, ~2x10^4 cells
were seeded into 24-well plates, washed three times with PBS and fixed for 20 min with 4% PFA at room temperature. PBS with 1% Triton X-100 was then added for 20 min. Following three washes with PBS, the cells were blocked for 1 h with 4% goat serum (diluted with PBS) at room temperature. The cells were then incubated with a rabbit monoclonal antibody against LC3 II (1:100; catalog no. ab48394; Abcam) at 4°C overnight. Alexa Fluor® 488 goat anti-rat IgG secondary antibody (1:400; Invitrogen; catalog no. O-6382; Thermo Fisher Scientific, Inc.) was then added for 2 h at room temperature in the dark. DAPI (300 nM) was used for nuclear staining for 15 min at room temperature. Images of ten randomly selected microscopic fields were captured using a confocal microscope (magnification, x2,000).

**Patient data analysis.** Patient data and gene expression datasets were obtained from the R2: Microarray analysis and visualization platform (http://hgserver1.amc.nl/cgi-bin/r2/main.cgi), which contains data from the ‘Tumour Neuroblastoma public Versteeg’, ‘Tumour Neuroblastoma-SEQC’ and ‘Tumour Neuroblastoma-Kocak’ datasets (28). These datasets contain mRNA expression data and no protein expression data. The Versteeg dataset contains 88 cases of neuroblastoma with tumor grade and gene variation. All prognosis analyses were performed with R2, and all data and P-values from a log-rank test were downloaded. Kaplan-Meier analysis was performed and the resulting survival curves were generated using GraphPad Prism 6.0 software (GraphPad Software, Inc., La Jolla, CA, USA). All cut-off values for generating high and low expression groups were determined using the online R2 database algorithm.

**Statistical analysis.** All data were analyzed using SPSS software (version 20.0; IBM Corp., Armonk, NY, USA). Quantitative data are presented as the mean ± standard deviation. One-way analysis of variance followed by Fisher’s least significant difference test was used to assess significant differences. P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Increased expression of TP53BP2 is associated with poor prognosis of patients with neuroblastoma.** To investigate whether TP53BP2 can be used as a prognostic indicator of neuroblastoma. A Kaplan-Meier analysis of progression-free survival for the Versteeg data indicated that an increased expression level of TP53BP2 is associated with a poor prognosis and a low expression level of TP53BP2 is associated with improved overall survival (Fig. 1A). Similar results were observed for the SEQC and Kocak data (Fig. 1B and C). In summary, all data indicated that an increased expression level of TP53BP2 is associated with a poor prognosis for patients with neuroblastoma.

Subsequently, RT-qPCR and western blot assays were performed to determine the expression of TP53BP2 in the neuroblastoma cell lines SK-N-AS, BE(2)-C, SK-N-DZ, SK-N-F1 and SHEP1. It was identified that TP53BP2 was expressed in all five cell lines (Fig. 1D and E). The aim of these experiments was to illustrate the involvement of TP53BP2 in the tumorigenesis of neuroblastoma (29).

Figure 2. Efficiency of TP53BP2 knockdown. The reverse transcription-quantitative polymerase chain reaction was used to determine the expression level of TP53BP2 in (A) BE(2)C and (B) SK-N-DZ cells following knockdown of TP53BP2. Western blot analysis was used to determine the expression level of TP53BP2 in (C) BE(2)C and (D) SK-N-DZ cells following knockdown of TP53BP2. Tubulin was used as the control. Quantification of the western blot data regarding TP53BP2 expression in (E) BE(2)C and (F) SK-N-DZ cells following knockdown of TP53BP2. Data are presented as the mean ± standard deviation. ***P<0.001. sh, short hairpin RNA; TP53BP2, tumor protein p53-binding protein 2.
Inhibition of TP53BP2 suppresses neuroblastoma cell colony formation in vitro. In numerous studies, a soft agar assay has been used as a human tumor stem-cell assay to investigate the ability of individual cancer cells to proliferate and form colonies (32). As presented in Fig. 4C and D, the role of TP53BP2 in neuroblastoma tumorigenesis was examined, which revealed that the colonies were smaller and significantly fewer in number for the TP53BP2-knockdown cells compared with for the controls (Fig. 4C and D). These results indicate that TP53BP2 can suppress neuroblastoma cell colony formation in vitro.

Inhibition of TP53BP2 induces neuroblastoma cell autophagy. Immunofluorescence assays were performed to detect the expression of LC3 II, which is a marker of autophagy (33). The results revealed that LC3 II expression increased markedly in the TP53BP2-knockdown cells compared with the controls (Fig. 5A). Furthermore, the expression levels of LC3B proteins in TP53BP2-knockdown and shGFP neuroblastoma cells were detected using western blot analysis. It was identified that the expression level of LC3 II was significantly increased in the TP53BP2-knockdown cells compared with in the controls (Fig. 5B-G). To confirm the occurrence of autophagy, RT-qPCR analysis revealed that the expression levels of ATG3, ATG5 and ATG7 were significantly upregulated in TP53BP2-knockdown cells (Fig. 5H and I). These results indicate that the inhibition of TP53BP2 upregulates the expression of LC3 II and induces autophagy.

Discussion
TP53BP2, also termed ASPP2, is a member of the ASPP family and cooperates with p53 to repress tumor growth (34). Previous
studies have reported that TP53BP2 serves a critical role in the tumorigenesis of different cancer types (12,13). TP53BP2 has been identified to be associated with susceptibility to gastric cancer (16). Furthermore, it has been demonstrated that TP53BP2 serves an important role in epithelial plasticity, which suppresses tumor metastasis (35). Previously, it has been reported that TP53BP2 is regulated by signal transducer and activator of transcription 1 to form part of the signaling pathway that suppresses tumors (36). In breast cancer, proliferation is inhibited and apoptosis is induced following knockdown of TP53BP2 (15). However, to the best of our knowledge, the role of TP53BP2 in neuroblastoma remains unknown. Therefore, the aim of the present study was to elucidate the role of TP53BP2 in neuroblastoma cells.

The results of the present study identified that the expression level of TP53BP2 was associated with the prognosis of patients with neuroblastoma. An increased expression level of TP53BP2 was identified to be associated with a worse prognosis. Furthermore, TP53BP2 was revealed to be expressed in all five neuroblastoma cell lines investigated, which suggests that TP53BP2 may be involved in the development of neuroblastoma. Subsequently, the effect of TP53BP2 knockdown on the proliferation of neuroblastoma cells was investigated. The results indicated that the proliferation of neuroblastoma cells was inhibited when TP53BP2 was down-regulated. In addition, BrdU assays confirmed an inhibition of proliferation following TP53BP2 knockdown. Furthermore, the results of the present study indicated that a downregulation of TP53BP2 suppresses the colony-formation capability of neuroblastoma cells in vitro. Notably, it was identified that a downregulation of TP53BP2 induces autophagy; indicated by an increased level of LC3 II (37-39). Subsequently, using western blot and RT-PCR analysis, the expression levels of autophagy-associated proteins, including LC3 II and ATG, were identified to increase following knockdown of TP53BP2.

In conclusion, the results of the present study indicated that TP53BP2 can regulate the proliferation and autophagy of neuroblastoma cells. However, the specific regulatory mechanisms of TP53BP2 were not determined and require further investigation. Another limitation of the present study was the absence of data regarding solid tumors, as there may be
differences between neuroblastoma cell lines and tumors. In summary, TP53BP2 may prevent autophagy and promote the proliferation of neuroblastoma.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Authors' contributions
YP performed the majority of the experiments and wrote the manuscript. LP participated in the cell experiments and statistical analysis. YZ assisted with the immunoblot assays and immunofluorescence staining. GL designed the study and helped to revise the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate
Not applicable.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Li T, Cui ZB, Ke XX, Tan J, Li FF, Li T, Wang XW and Cui HJ: Essential role for p53 and caspase-9 in DNA damaging drug-induced apoptosis in neuroblastoma IMR32 cells. DNA Cell Biol 30: 1045-1050, 2011.
2. Li T, Wang L, Ke XX, Gong XY, Han JH, Hao XW, Xu M, Xiang Z, Cui ZB and Cui H: DNA-damaging drug-induced apoptosis sensitized by N-myc in neuroblastoma cells. Cell Biol Int 36: 331-337, 2012.
3. Askim FB and Perlman EJ: Neuroblastoma and peripheral neuroectodermal tumors. Am J Clin Pathol 109 (4 Suppl 1): S23-S30, 1998.
4. Shah S and Ravindranath Y: Neuroblastoma. Indian J Pediatr 65: 691-705, 1998.
5. Bessho F: Incidence of neuroblastoma. Lancet 353: 70, 1999.
6. Sridhar S, Al-Moalem B, Kamal H, Terille M and Stallings RL: New insights into the genetics of neuroblastoma. Mol Diagn Ther 17: 63-69, 2013.
7. Beckwith JB and Martin RF: Observations on the histopathology of neuroblastomas. J Pediatr Surg 3: 106-110, 1968.
8. Zhu S, Yan X, Xiang Z, Ding HF and Cui H: Leflunomide reduces proliferation and induces apoptosis in neuroblastoma cells in vitro and in vivo. PLoS One 8: e17155, 2013.
9. Cheung NK and Dyer MA: Neuroblastoma: Developmental biology, cancer genomics and immunotherapy, Nat Rev Cancer 13: 397-411, 2013.
10. Morgenstern DA, Baruchel S and Irwin MS: Current and future strategies for relapsed neuroblastoma: Challenges on the road to precision therapy. J Pediatr Hematol Oncol 35: 337-347, 2013.
11. Liu L, Shi Y, Guo X, Wang S, Ouyang Y, Wu M, Liu D, Liu N, Zheng J and Chen D: CHOP mediates ASPP2-induced autophagic apoptosis in hepatoma cells by releasing Beclin-1 from Bcl-2 and inducing nuclear translocation of Bcl-2. Cell Death Dis 5: e1123, 2014.
12. Ma L, Chen ZM, Li XY, Wang XJ, Shou JX and Fu XD: Nucleostemin and ASPP2 expression is correlated with pituitary adenoma proliferation. Oncol Lett 6: 1313-1318, 2013.
13. Vives V, Slee EA and Lu X: ASPP2: A gene that controls life and death in vivo. Cell Cycle 5: 2187-2190, 2006.
14. Tondella L, Koch S, Salter V, Pagotto A, Doonbeeja JB, Feller SM, Ratnayaka I, Zhong S, Goldin RD, Lozano G, et al: ASPP2 suppresses squamous cell carcinoma via RelA/p65-mediated repression of p63. Proc Natl Acad Sci USA 110: 17969-17974, 2013.
15. Song Q, Song J, Wang Q, Ma Y, Sun N, Ma J, Chen Q, Xia G, Huo Y, Yang L and Li B: MiR-548d-3p/TP53BP2 axis regulates the proliferation and apoptosis of breast cancer cells. Cancer Med 5: 315-324, 2016.
16. Ju H, Lee KA, Yang M, Kim HJ, Kang CP, Sohn TS, Rhee JC, Kang C and Kim JW: TP53BP2 locus is associated with gastric cancer susceptibility. Int J Cancer 117: 957-960, 2005.
17. Bennet DE, Campobasso J, Diniz V, Diaz D, Latorre ML, Urteger AJ and Todaro LB: Autophagy: Friend or foe in breast cancer development, progression, and treatment. Int J Breast Cancer 2011: 590592, 2011.
18. Fimia GM, Kroemer G and Piacentini M: Molecular mechanisms of autophagic apoptosis. Cell Death Differ 20: 1-12, 2013.
19. Klionsky DJ: Autophagy: From phenomenology to molecular understanding in less than a decade. Nat Rev Mol Cell Biol 8: 931-937, 2007.
20. Mehrpour M, Esclatine A, Beau I and Codogno P: Overview of macroautophagy regulation in mammalian cells. Cell Res 20: 748-762, 2010.
21. Rubinsztein DC, Codogno P and Levine B: Autophagy modulation as a potential therapeutic target for diverse diseases. Nat Rev Drug Discov 11: 709-730, 2012.
22. Mizushima N and Komatsu M: Autophagy: Renovation of cells and tissues. Cell 147: 728-741, 2011.
23. Wirawan E, Vanden Berghe T, Lippens S, Agostinis P and Vandenameele P: Autophagy: For better or for worse. Cell Res 22: 43-61, 2012.
24. Levine B, Mizushima N and Virgin HW: Autophagy in immunity and inflammation. Nature 469: 323-335, 2011.
25. Towers CG and Thorburn A: Therapeutic targeting of autophagy. EBioMedicine 14: 15-23, 2016.
26. Han X, Liu JX and Li XZ: Salvianolic acid B inhibits autophagy and protects starving cardiac myocytes. Acta Pharmacol Sin 32: 38-44, 2011.
27. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
28. Molenaar JJ, Koster J, Zwijnenburg DA, van Sluis P, Valenti LJ, van der Ploeg I, Hamdi M, van Nes J, Westerman BA, van Arkel J, et al: Sequencing of neuroblastoma identifies chromothripsis and defects in neurotogenins genes. Nature 483: 589-593, 2012.
29. Yang R, Wu Y, Zou J, Zhou J, Wang M, Hao X and Cui H: The Hippo transducer TAZ promotes cell proliferation and tumor formation of neuroblastoma cells through EGFR pathway. Oncotarget 7: 36255-36265, 2016.
30. Zhang D, Wang F, Pang Y, Ke XX, Zhu S, Zhao E, Zhang K, Chen L and Cui H: Down-regulation of CHERP inhibits neuroblastoma cell proliferation and induces apoptosis through ER stress induction. Oncotarget 8: 80956-80970, 2017.
31. Yang L, Huang M, Tan J, Hou J, He J, Wang F, Cui H and Yi L: Transcriptional co-activator with PDZ-binding motif overexpression promotes cell proliferation and transcriptional co-activator with PDZ-binding motif deficiency induces cells cycle arrest in neuroblastoma. Oncol Lett 13: 4295-4301, 2017.
32. Agrez MV, Kovach JS and Lieber MM: Cell aggregates in the soft agar ‘human tumour stem-cell assay’. Br J Cancer 46: 880-887, 1982.
33. Ke XX, Zhang D, Zhu S, Xia Q, Xiang Z and Cui H: Inhibition of HSK9 methyltransferase G9a repressed cell proliferation and induced autophagy in neuroblastoma cells. PLoS One 9: e106962, 2014.
34. Vives V, Su J, Zhong S, Ratnayaka I, Slee E, Goldin R and Lu X: ASPP2 is a haploinsufficient tumor suppressor that cooperates with p53 to suppress tumor growth. Genes Dev 20: 1262-1267, 2006.

35. Wang Y, Bu F, Royer C, Serres S, Larkin JR, Soto MS, Sibson NR, Salter V, Fritzschke F, Turnquist C, et al: ASPP2 controls epithelial plasticity and inhibits metastasis through β-catenin-dependent regulation of ZEB1. Nat Cell Biol 16: 1092-1104, 2014.

36. Turnquist C, Wang Y, Severson DT, Zhong S, Sun B, Ma J, Constaninescu SN, Ansorge O, Stolp HB, Molnár Z, et al: STAT1-induced ASPP2 transcription identifies a link between neuroinflammation, cell polarity, and tumor suppression. Proc Natl Acad Sci USA 111: 9834-9839, 2014.

37. Mai S, Muster B, Bereiter-Hahn J and Jendrach M: Autophagy proteins LC3B, ATG5 and ATG12 participate in quality control after mitochondrial damage and influence lifespan. Autophagy 8: 47-62, 2012.

38. Hale AN, Ledbetter DJ, Gawriluk TR and Rucker EB III: Autophagy: Regulation and role in development. Autophagy 9: 951-972, 2013.

39. Zhao H, Yang M, Zhao J, Wang J, Zhang Y and Zhang Q: High expression of LC3B is associated with progression and poor outcome in triple-negative breast cancer. Med Oncol 30: 475, 2013.