Int J Clin Exp Pathol 2017;10(7):8023-8030
www.ijcep.com /ISSN:1936-2625/IJCEP0046500

Original Article

Effects of ASPP2 on proliferation and apoptosis of malignant spinal tumor cells

Wei Huang[1], Xinzhi Li[2], Lin Cai[1]

[1]*Department of Orthopedics, Zhongnan Hospital of Wuhan University, Wuhan University, Wuhan, Hubei, China;*
[2]*Department of Orthopedics, Renhe Hospital of Three Gorges University, Yichang, Hubei, China*

Received December 13, 2016; Accepted December 27, 2016; Epub July 1, 2017; Published July 15, 2017

Abstract: Malignant spinal tumors have rapid progression and destruct spines or other tissues, leading to metastasis of peripheral organs, causing high difficulty for surgery, recurrence and worse prognosis, thus severely affecting patient life quality and survival period. Apoptosis stimulating protein 2 of p53 (ASSP2) is one member of p53 binding protein family pro-apoptotic member, and can enhance apoptotic activity via modulating p53 pathway. Previous study found critical roles of ASPP2 in occurrence and progression of tumors, whilst the functional role of mechanism of ASPP2 on malignant spinal tumor cells has not been illustrated. Malignant spinal tumor tissue and adjacent tissues were collected for testing ASPP2 mRNA and protein expression in real-time PCR and Western blot. ASPP2 over-expression vector was used to transfect tumor cells, whilst MTT assay was employed for tumor proliferation, followed by p53 expression in Western blot. Caspase 3 activity assay was employed for testing the effect on cell apoptosis. Flow cytometry was employed for tumor cell apoptosis. Real-time PCR tested expressional change of Bcl2 and Bax. ASPP2 mRNA/protein level was significantly depressed in malignant spinal tumor tissues ($P<0.05$ compared to adjacent tissues). ASPP2-overexpression vector transfection tumor cells increased apoptosis and inhibited proliferation, accompanied with lower Bcl-2, higher Bax, Caspase 3 and p53 ($P<0.05$ compared to control group). ASPP2 is down-regulated in malignant spinal tumor tissues. ASPP2 can inhibit malignant spinal tumor cell proliferation via mediating p53 expression for cell apoptosis, thus can work as one molecular target for tumor diagnosis and prognostic analysis.

Keywords: Malignant spinal tumor, ASPP2, p53, proliferation, apoptosis

Introduction

Spinal tumor occupies about 10% of all bone tumors, with different subtypes including osteosarcoma or bone-like cancer [1, 2]. Spinal tumors can be divided into three classes: primary benign spinal tumors; primary malignant spinal tumors and metastatic spinal tumors [3]. Malignant spinal tumor has rapid progression, tendency for invasion/metastasis, and can lead to spine destruction or affecting other tissues, causing invasion of tumor cells toward peripheral tissues [4]. Early diagnosis of spinal tumor is critical. However, due to insidious occurrence of malignant spinal tumor, which typically manifests as back pain, nerve compression, body immobility and focal lesion, featured clinical manifestation is lacked, causing difficulty in early diagnosis and higher misdiagnosis [5, 6]. Most spinal tumor tissues are already at terminal stage when being diagnosis, causing major difficulty for treatment and affecting efficiency [7]. Surgery for malignant spinal tumor has higher difficulty and recurrent rate, causing worse prognosis and unfavorable survival period and life quality [8]. Pathogenesis and invasion mechanism of malignant spinal tumor are complicated, involving multiple factors including genetics, environment and biology [9]. The inhibition of tumor invasion and metastasis is critical for improving prognosis of malignant spinal tumors. Therefore, the illustration of pathogenesis for spinal tumor and the identification of molecular diagnostic target are important for early diagnosis of malignant spinal tumors and for improving patient's prognosis.

Apoptosis stimulating protein of p53 (ASPP) family is one group of apoptosis regulatory pro-

teins [10]. Currently, ASPP family has the most complete composition in p53 gene regulation, including ASPP1, ASPP2 and iASPP [11]. ASPP family includes sufficient anchor proteins, SH3 domain and abundant glutamic acid structural domains [12]. ASPP family can bind with p53 protein to facilitate its cell apoptotic function [13]. As one tumor suppressor gene, p53 can induce tumor cell apoptosis thus inhibiting tumor progression [14]. Therefore, ASPP family is important for regulatory tumor function. Among all its family members, ASPP2 is the most powerful one, and exerts functions among multiple tumors [15, 16]. The function and mechanism of ASPP2 for malignant spinal tumors, however, have not been illustrated.

Materials and methods

General information

Five primary malignant spinal tumor patients who received pathology examination from January 2015 to August 2016 in Zhongnan Hospital of Wuhan University were recruited. All patients received surgery for radical resection. Patients aged from 31 to 56 years (average age =37.2±5.6 years). Tissue samples were collected during the surgery for pathology examination and subtyping/staging. All cases were confirmed as primary malignant spinal tumor by pathology examination. Tumor lesion sites include one cervical spine, one thoracic spine and three cases at lumber segment. Both tumor and adjacent tissues were collected during the surgery for -80°C frozen. This study has been approved by the ethical committee of Zhongnan Hospital of Wuhan University. All subjects have signed informed consents.

Inclusive and exclusive criteria

Inclusive criteria [5, 6]: Those patients confirmed as primary malignant spinal tumors by pathology; Primary tumor onset without surgery, chemo-or radio-therapy before. Exclusive criteria included those have received surgeries before, or chemo-/radio-therapy; Patients complicated with other major diseases including infection, other cancers, severe liver/kidney disorder, pulmonary fibrosis, bone metabolic disorder, secondary renal hypertension, systemic immune disease and malignant tumor complication; Plus those who were unwilling to cooperate this study.

Major reagent and equipment

DMEM culture medium, fetal bovine serum (FBS) and streptomycin-penicillin were purchased from Hyclone (US). DMSO and MTT powders were purchased from Gibco (US). Trypsin-EDTA lysis buffer was purchased from Sigma (US). PVDF membrane was purchased from Pall Life Sciences (US). Western blotting reagent was purchased from Beyotime (China). ECL reagent was purchased from Amersham Biosciences (US). Rabbit anti-human ASPP monoclonal antibody, rabbit anti-human p53 monoclonal antibody and goat anti-rabbit horseradish peroxidase (HRP)-conjugated IgG secondary antibody were all purchased from Cell Signaling (US). RNA extraction kits and reverse transcription kit were purchased from Axygen (US). Labsystem Version 1.3.1 microplate reader was purchased from Bio-Rad (US). Lipo2000 transfection reagent was purchased from Life (US). BD FACSCalibur flow cytometry was purchased from BD (US). ABI7700 Fast fluorescent quantitative PCR cycler was purchased from ABI (US). THermo Scientific Forma CO_2 incubator was purchased from Thermo (US). Ultrapure workstation was purchased from Sutai Engineering (China). Other common reagents were purchased from Sangon (China).

Primary culture of spinal tumor cells and grouping

Malignant spinal tumor tissues were digested in trypsin, followed by 1,000 rpm for 5 min to discard the supernatant. Cell precipitation was re-suspended in 4 ml DMEM medium (containing 100 U/ml penicillin, 100 μg/ml streptomycin) with 10% FBS. Cells were cultured in a humidified chamber with 5% CO_2 at 37°C. Cells at 3rd to 8th log-growth phase were selected and randomly assigned into control, empty plasmid and ASPP2 groups.

Transfection of pcDNA 3.1-ASPP2 plasmid into cells

Trizol reagent was used to extract RNA from cultured tumor cells. Using total RNA as the template, DNA transcription was performed. Primers were designed by PrimerPremier6.0 and synthesized by Sangon (China). Primer sequences were: ASPP2-forward 5'-TGACT CTTCT GCGGC CTGAT-3'; reverse 5'-TGACT CTTCT GCGGC CTGAT-3'. PCR amplification was

Table 1. Primer sequence

Target gene	Forward primer 5'-3'	Reverse primer 5'-3'
GAPDH	AGTACCAGTCTGTTGCTGG	TAATAGACCCGGATGTCTGGT
ASPP2	CTCATCGTAACAATGG	GCACCGTTCTTAGCG
Bcl2	TAAGAGGAACGGAATG	ACATCATCTATTCTCT
Bax	CAGCTCCTACCAGCTTAAG	GCACGATTGACTGCTTTGTGA

performed using cDNA as the template under the following conditions: 95°C for 2 min, and 50°C for 1 min, followed by 35 cycles each containing 94°C for 30 s, 60°C for 50 s and 72°C for 35 s. PCR products were recycled by gel extraction kit, and were ligated to pcDNA3.1 vector at 3:1 ratio. Ligation was performed at 4°C for 16 h. JM109 competent cells were prepared in LB plate and LB medium, with the transfection of clonal plasmids. pcDNA3.1-ASPP2 plasmid was concentrated and quantified. Cancer cells at log growth phase were collected and adjusted to 3×10^6 per ml in 6-well plate. Cells were incubated in a 37°C chamber with 5% CO_2 for 12 h until reaching 70%~80% confluence. 5 μl lipo2000 reagent was diluted in 200 μl serum-free DMEM medium for 15 min room temperature incubation. pcDNA3.1-ASPP2 and pcDNA3.1 plasmids were mixed in 200 μl serum-free medium for 15 min room temperature incubation. Lipo2000 mixture was then mixed with pcDNA3.1-ASPP2 dilutions for 30 min room temperature incubation. Serum was removed from cells in 6-well plate, followed by PBS rinsing and the addition of 1.6 ml serum-free culture medium. Cells were then kept in a humidified chamber with 5% CO_2 at 37°C for 6 h, followed by the application of serum-containing medium for further experiments.

Real-time PCR for ASPP2, Bcl2 and Bax gene expression in tumor tissues and cells

Trizol reagent was used to extract RNA from tumor or adjacent tissues and tumor cells. Reverse transcription was performed according to the manual instruction (Axygen, US), using primers designed by PrimerPrimer6.0 and synthesized by Invitrogen (China) as shown in **Table 1**. Real-time PCR was performed under the following conditions: 55°C for 1 min, followed by 35 cycles each containing 92°C for 30 s, 58°C for 45 s and 72°C for 35 s. Data were collected and calculated for CT values of all samples and standards based on fluorescent quantification using GAPDH as the internal control. Standard curve was firstly plotted using CT values of standards, followed by semi-quantitative analysis by $2^{-\Delta Ct}$ method.

Western blot for ASPP2 and p53 protein expression in tissues and cells

Proteins from tumor or adjacent tissues and DU145 proteins were firstly extracted. In brief, RIPA lysis buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 2 μg/ml Aprotinin, 2 μg/ml Leupeptin, 1 mM PMSF, 1.5 mM EDTA and 1 mM NaVandade) was used to lyse cells on ice for 15~30 min, followed by ultrasound rupture (5 s×4) and centrifugation (4°C, 10 000 g, 15 min). Supernatants were saved and quantified by Bradford approach, and were stored at -20°C for further Western blotting. Proteins were then separated using 10% SDS-PAGE gel, and were transferred to PVDF membrane using semi-dry method (100 mA for 1.5 h). Non-specific background was removed by 5% defatted milk powder at room temperature for 2 h, followed by the addition of anti-ASPP2 monoclonal antibody (1:1000 dilution), or anti-p53 monoclonal antibody (1:1500) in 4°C overnight incubation. On the next day, the membrane was rinsed in PBST, and incubated with goat anti-rabbit secondary antibody (1:2000) for 30 min incubation. After PBST rinsing, ECL reagent was used to develop the membrane for 1 min, followed by exposure under X-ray for observing results. Protein imaging analysis system and Quantity One software were used to scan X-ray films for observing band density. Each experiment was repeated for four times (N=4) for statistical analysis.

MTT assay for cell proliferation

Tumor cells at log-phase were counted and seeded into 96-well plate at 5×10^3 cells per well. Cells were then randomly divided into control, empty plasmid group and ASPP2 group as abovementioned. After 48-hour incubation, 20 μl sterile MTT solution was then added into each test well in triplicates. With 4 h continuous culture, the supernatant was completely removed, with the addition of 150 μl DMSO for 10 min vortex until the complete resolving of crystal violet. Absorbance (A) values was measured at 570 nm in a microplate reader. The proliferation rate was calculated in each group.

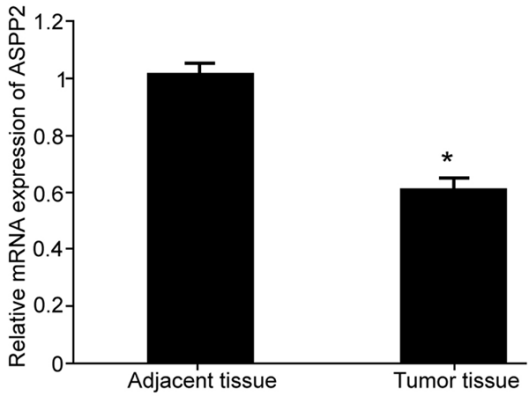

Figure 1. ASPP2 mRNA expression in malignant spinal tumor and adjacent tissues. *, P<0.05 compared to adjacent tissues.

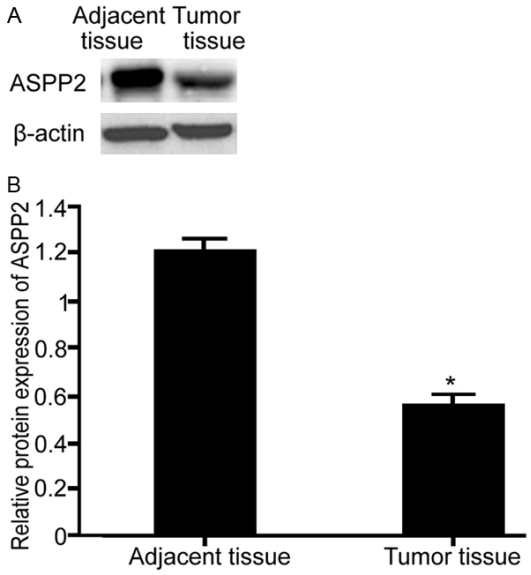

Figure 2. ASPP 2 protein expression in malignant spinal tumor tissues and adjacent tissues. A. Western blot for ASPP2 protein expression in spinal tumor tissues and adjacent tissues; B. ASPP2 protein expression analysis between malignant spinal tumor tissues and adjacent tissues. *, P<0.05 compared to adjacent tissues.

Each experiment was repeated in triplicates for statistical analysis.

Caspase3 activity assay

Caspase 3 activity in all groups of cells was evaluated using test kit. In brief, cells were digested by trypsin, and were centrifuged at 600 g for 5 min under 4°C. The supernatant was discarded, followed by the addition of cell lysis buffer and iced incubation for 15 min. The

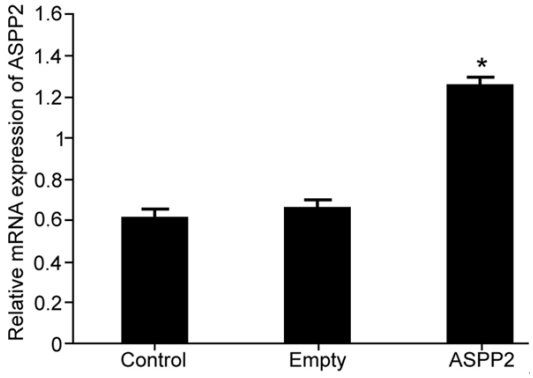

Figure 3. Effects of ASPP2 transfection on gene expression in tumor cells. *, P<0.05 compared to control group.

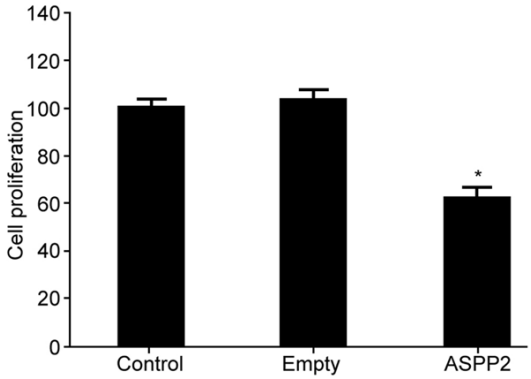

Figure 4. Effects of ASPP2 on proliferation of spinal tumor cells. *, P<0.05 compared to control group.

mixture was then centrifuged at 20,000 g for 5 min under 4°C, followed by the addition of 2 mM Ac-DECD-pNA. OD values at 450 nm wavelength were measured to reflect caspase3 activity.

Flow cytometry for effects of ASPP2 on tumor cell apoptosis

Tumor cells were digested, counted and inoculated into 50 ml culture flask at 5×10^5 per ml density. Cells were randomly assigned into control, empty plasmid and ASPP2 groups. Cells were digested, counted and collected at 2×10^6 per ml density. Cells were washed in 1×PBS at 1000 rpm for 5 min, and were fixed in pre-cold 75% ethanol at 4°C overnight. After discarding ethanol, cells were washed in 1×PBS for 5 min by 1000 rpm centrifugation. Cells were then resuspended into 0.8 ml mixture containing 1×PBS and 1% BSA, with addition of 100 μg/ml PI dye (3.8% Sodium Citrate, pH7.0). RnaseA

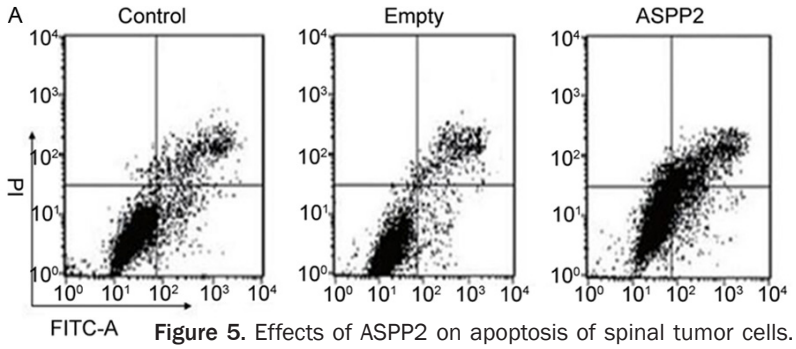

Figure 5. Effects of ASPP2 on apoptosis of spinal tumor cells. A. Flow cytometry for the effect of ASPP2 over-expression on apoptosis of spinal tumor cells; B. ASPP2 over-expression and spinal tumor tissue apoptosis: an analysis. *, P<0.05 compared to control group.

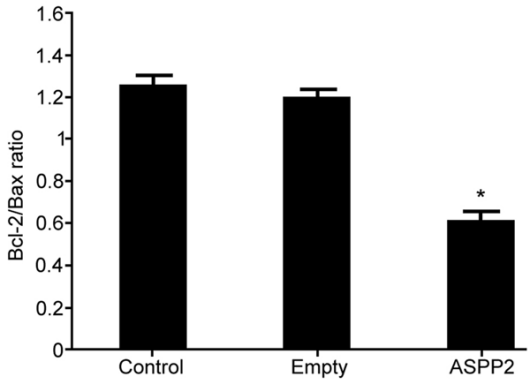

Figure 6. Effects of ASPP2 on Bcl2/Bax ratio in spinal tumor cells. *, P<0.05 compared to control group.

(10 mg/ml) was then added for 37°C dark incubation for 30 min. Flow cytometry was used to collect data, which were analyzed by FCS-Express 3.0 software.

Statistical processing

SPSS 16.0 software was used for data analysis. Measurement data were presented as mean ± standard deviation (SD). Comparison of multiple groups was performed using one-way analysis of variance. Between-group analysis was performed by LSD test. A statistical significance was defined when P<0.05.

Results

ASPP2 mRNA expression in malignant spinal tumor tissues

Real-time PCR was used to test ASPP2 mRNA expression in malignant spinal tumor tissues and adjacent tissues. Results fund significantly decreased ASPP2 mRNA expression in malignant spinal tumor tissues (P<0.05 compared to tumor adjacent tissues, **Figure 1**).

ASPP2 protein expression in malignant spinal tumor tissues

Western blot was used to test ASPP2 protein expression in malignant spinal tumor and adjacent tissues. Results showed significantly decreased ASPP2 protein expression in malignant spinal tumor tissues (P<0.05 compared to adjacent tissues, **Figure 2**).

ASPP2 expression in malignant spinal tumor tissues

Real-time PCR was used to test ASPP2 expression change in malignant spinal tumor cells after ASPP2 plasmid transfection. Results showed significantly elevated ASPP2 expression after transfecting pcDNA3.1-ASPP2 plasmid (P<0.05 compared to control group, **Figure 3**).

Effects of ASPP2 on proliferation of spinal tumor cell proliferation

MTT assay was used to test the effect of ASPP2 over-expression on proliferation of spinal tumor cells. Results showed that elevated ASPP2 expression inhibited proliferation of spinal tumor cells (P<0.05 compared to control group, **Figure 4**).

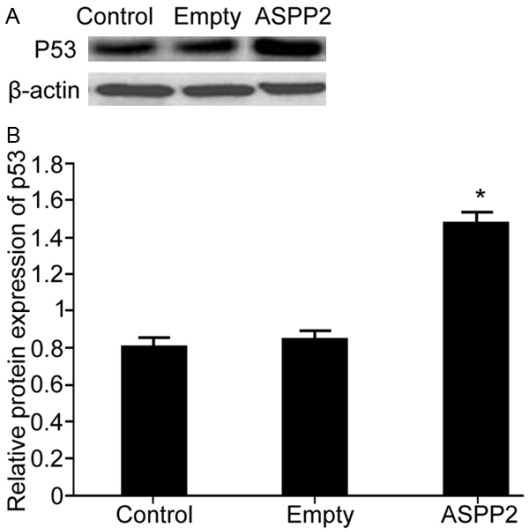

Figure 7. Effects of ASPP2 on p53 expression in spinal tumor cells. A. Western blot for p53 protein expression in spinal tumor cells by ASPP2; B. p53 protein expression analysis by ASPP2. *, P<0.05 compared to control group.

Effects of ASPP2 on apoptosis of spinal tumor cell proliferation

Flow cytometry was used to analyze the effect of ASPP2 over-expression on apoptosis of spinal tumor cells. Results showed that elevated ASPP2 expression facilitated spinal tumor cell apoptosis (P<0.05 compared to control group, **Figure 5**).

Effects of ASPP2 on apoptosis genes Bcl2 and Bax in spinal tumor cells

Real-time PCR was used to test the effect of ASPP2 over-expression in spinal tumor cells on expression of anti-apoptosis gene Bcl-2 and apoptosis gene Bax. Results demonstrated that elevated ASPP2 expression inhibited the expression of anti-apoptotic gene Bcl2 expression, and facilitated pro-apoptotic gene Bax expression, thus lowering Bcl2/Bax ratio (P<0.05 compared to control group, **Figure 6**).

Effects of ASPP2 on p53 expression in spinal tumor cells

Western blot was used to analyze the effect of ASPP2 over-expression on tumor cell p53 expression. Results showed that elevated ASPP2 expression facilitated tumor suppressor gene p53 expression (P<0.05 compared to control group, **Figure 7**).

Discussion

Malignant spine tumor lesion is surrounded by abundant of micro-vessels, making it predisposed to peripheral tissues, causing tissue edema and invasion, making its high malignancy and easy for metastasis. Moreover, due to incomplete resection of tumor during surgery or residual tumors, high recurrent rate exists [4]. Therefore it is critical to explore the identification of pathogenesis and treatment target of spinal malignant tumors for improving patient prognosis. ASSP2 is coded by TP53BP2 gene, and is composed of 1128 amino acids. After binding between carboxyl terminal of ASPP2 protein and p53, the latter of which can be induced for downstream gene transcription [17, 18]. ASPP2 participates in injury related stress, cell growth, proliferation and regulation of apoptosis, thus having significant anti-tumor effects [19]. The expression of ASPP2 in malignant spinal tumor has not been reported yet. This study revealed significantly down-regulated mRNA or protein level of ASPP2 in primary spinal tumors, indicating consistent expression pattern of ASPP2 as in other tumors. Therefore, ASPP2 also exerts tumor suppressing effects in malignant spinal tumors.

Further *in vitro* culture of primary tumor cells, they were transfected with ASPP2 to establish an over-expression model for illustrating the role of ASPP2 in malignant spinal tumors. Results confirmed the over-expression of ASPP2 in malignant spinal tumor cells, whose proliferation potency was inhibited whilst tumor inducing potency was enhanced. When cells have abnormality, ASPP2 can selectively activate pro-apoptotic gene mainly via specific binding onto p53 protein. P53-induced cell signal transduction pathway plays an important role in mediating normal cell activity, and is the most relevant gene for tumor ever been identified. It can bind with certain locus of genes to work as one special transcriptional factor to activate p21 gene transcription, to arrest cell cycle, to inhibit helicase activity and interact with replication factor A in DNA replication and repairmen. P53 protein can initiate cell suicide during apoptotic process, thus preventing cell malignancy [20, 21]. ASPP2 bind with p53 to activate downstream Caspase family member. Caspase 3 is the most powerful member in Caspase family for apoptosis induction. The

elevation of its activation can lead to irreversible cell damage [22]. ASPP2 is one E2F regulatory protein, and can facilitate cell apoptosis via activating p53 protein-induced trans-activated pro-apoptotic gene Bax [23]. Bcl-2 inhibits p53-induced apoptosis via forming dimer with Bax. When Bax has relatively higher expression than Bcl-2, Bax-homodimer number is increased to facilitate cell death. On the other hand, if Bcl-2 relative level is higher than Bax, Bcl-2/Bax heterodimer formation is facilitated, thus inhibiting cell death [24, 25]. This study demonstrated that the over-expression of ASPP2 in spinal tumor cells could facilitate p53 expression or Bax expression or Caspase 3 activity, and decrease Bcl2 expression or Bcl2/Bax ratio.

Conclusion

ASPP2 is down-regulated in malignant spinal tumor patients. ASPP2 can modulate cell apoptosis via mediating p53 expression, and can inhibit proliferation of malignant spinal cells, thus working as one potential molecular target for tumor diagnosis and prognostic analysis.

Acknowledgements

This work was supported by National Natural Science Foundation of China (NO. 81571811).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Lin Cai, Department of Orthopedics, Zhongnan Hospital of Wuhan University, Wuhan University, 169 Donghu Road, Wuchang District, Wuhan 430071, Hubei, China. Tel: +86-27-67813116; Fax: +86-27-67813116; E-mail: lincaiop@163.com

References

[1] Nagpal AP, Chandra S and Goel S. Large retroperitoneal extraosseous osteosarcoma invading into the spine: a case report. Indian J Surg Oncol 2016; 7: 464-466.

[2] Guzik G. The correspondence between magnetic resonance images and the clinical and intraoperative status of patients with spinal tumors. Curr Med Imaging Rev 2016; 12: 149-155.

[3] Landi A, Innocenzi G, Grasso G, Meschini A, Fabbiano F, Castri P and Delfini R. Diagnostic potential of the diffusion tensor tractography with fractional anisotropy in the diagnosis and treatment of cervical spondylotic and posttraumatic myelopathy. Surg Neurol Int 2016; 7: S705-S707.

[4] Johanns TM, Miller CA, Dorward IG, Tsien C, Chang E, Perry A, Uppaluri R, Ferguson C, Schmidt RE, Dahiya S, Ansstas G, Mardis ER and Dunn GP. Immunogenomics of hypermutated glioblastoma: a patient with germline POLE deficiency treated with checkpoint blockade immunotherapy. Cancer Discov 2016; 6: 1230-1236.

[5] Ahmed R, Menezes AH and Torner JC. Role of resection and adjuvant therapy in long-term disease outcomes for low-grade pediatric intramedullary spinal cord tumors. J Neurosurg Pediatr 2016; 18: 594-601.

[6] Ito K, Aoyama T, Nakamura T, Hanaoka Y, Horiuchi T and Hongo K. Novel dural incision and closure procedure for preventing postoperative cerebrospinal fluid leakage during the surgical removal of dumbbell-shaped spinal tumors: technical note. J Neurosurg Spine 2016; 25: 620-625.

[7] Vasudeva VS, Ropper AE, Rodriguez S, Wu KC and Chi JH. Contralateral osteotomy of the pedicle and posterolateral elements for en bloc resection: a technique for oncological resection of posterolateral spinal tumors. J Neurosurg Spine 2016; 1-7.

[8] Matsumoto Y, Matsunobu T, Harimaya K, Kawaguchi K, Hayashida M, Okada S, Doi T and Iwamoto Y. Bone and soft tissue tumors presenting as sciatic notch dumbbell masses: A critical differential diagnosis of sciatica. World J Clin Oncol 2016; 7: 414-419.

[9] Song Z, Xiong B, Zheng H, Manyande A, Guan X, Cao F, Ren L, Zhou Y, Ye D and Tian Y. STAT1 as a downstream mediator of ERK signaling contributes to bone cancer pain by regulating MHC II expression in spinal microglia. Brain Behav Immun 2017; 60: 161-173.

[10] Liu Z, Zang Y, Qiao L, Liu K, Ouyang Y, Zhang Y and Chen D. ASPP2 involvement in p53-mediated HIV-1 envelope glycoprotein gp120 neurotoxicity in mice cerebrocortical neurons. Sci Rep 2016; 6: 33378.

[11] Bromley D, Bauer MR, Fersht AR and Daggett V. An in silico algorithm for identifying stabilizing pockets in proteins: test case, the Y220C mutant of the p53 tumor suppressor protein. Protein Eng Des Sel 2016; 29: 377-90.

[12] Chen XL, Bai YJ, Hu QR, Li SS, Huang LZ, Li XX. Small interfering RNA targeted to ASPP2 promotes progression of experimental proliferative vitreoretinopathy. Mediators Inflamm 2016; 2016: 7920631.

[13] Liu H, Chen F, Zhang L, Zhou Q, Gui S and Wang Y. A novel all-trans retinoic acid derivative 4-amino2trifluoromethyl-phenyl retinate inhibits the proliferation of human hepatocellular carcinoma HepG2 cells by inducing G0/G1 cell cycle arrest and apoptosis via upregulation of p53 and ASPP1 and downregulation of iASPP. Oncol Rep 2016; 36: 333-41.

[14] Yamaguchi R, Yamamoto T, Sakamoto A, Ishimaru Y, Narahara S, Sugiuchi H and Yamaguchi Y. A protease-activated receptor 2 agonist (AC-264613) suppresses interferon regulatory factor 5 and decreases interleukin-12p40 production by lipopolysaccharide-stimulated macrophages: Role of p53. Cell Biol Int 2016; 40: 629-41.

[15] Shao LN, Zhu BS, Xing CG, Yang XD, Young W and Cao JP. Effects of autophagy regulation of tumor-associated macrophages on radiosensitivity of colorectal cancer cells. Mol Med Rep 2016; 13: 2661-70.

[16] Hollmann G, Linden R, Giangrande A and Allodi S. Increased p53 and decreased p21 accompany apoptosis induced by ultraviolet radiation in the nervous system of a crustacean. Aquat Toxicol 2016; 173: 1-8.

[17] Mak VC, Lee L, Siu MK, Wong OG, Lu X, Ngan HY, Wong ES and Cheung AN. Downregulation of ASPP2 in choriocarcinoma contributes to increased migratory potential through Src signaling pathway activation. Carcinogenesis 2013; 34: 2170-7.

[18] Kim H, Claps G, Moller A, Bowtell D, Lu X and Ronai ZA. Siah2 regulates tight junction integrity and cell polarity through control of ASPP2 stability. Oncogene 2014; 33: 2004-10.

[19] Meng WD, Chu RX, Wang BZ, Wang LP, Ma LL and Wang LX. Helicobacter pylori infection and expressions of apoptosis-related proteins p53, ASPP2 and iASPP in gastric cancer and precancerous lesions. Pathol Biol (Paris) 2013; 61: 199-202.

[20] Rotem-Bamberger S, Katz C and Friedler A. Regulation of ASPP2 interaction with p53 core domain by an intramolecular autoinhibitory mechanism. PLoS One 2013; 8: e58470.

[21] Li Y and Liu S. Direct evidence for the involvement of TP53 in the proliferation, migration and invasion of gastric carcinoma cells. Med One 2016; 1: 4.

[22] Wang Y, Godin-Heymann N, Dan Wang X, Bergamaschi D, Llanos S and Lu X. ASPP1 and ASPP2 bind active RAS, potentiate RAS signalling and enhance p53 activity in cancer cells. Cell Death Differ 2013; 20: 525-34.

[23] Choudhary AK and Devi RS. Effects of aspartame on hsp70, bcl-2 and bax expression in immune organs of Wistar albino rats. J Biomed Res 2016; 30: 427-435.

[24] Jiang Z, Liu X, Chang K, Liu X and Xiong J. Allyl isothiocyanate inhibits the proliferation of renal carcinoma cell line GRC-1 by inducing an imbalance between bcl2 and bax. Med Sci Monit 2016; 22: 4283-4288.

[25] Zheng LC, Yang MD, Kuo CL, Lin CH, Fan MJ, Chou YC, Lu HF, Huang WW, Peng SF and Chung JG. Norcantharidin-induced apoptosis of AGS human gastric cancer cells through reactive oxygen species production, and caspase-and mitochondria-dependent signaling pathways. Anticancer Res 2016; 36: 6031-6042.