

ORIGINAL ARTICLE

Downregulation of iASPP Expression Suppresses Proliferation, Invasion and Increases Chemosensitivity to Paclitaxel of Head and Neck Squamous Cell Carcinoma *In Vitro*

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Key words: human head and neck squamous cell carcinoma; iASPP; chemosensitivity; paclitaxel

Objective Our previous study has revealed that iASPP is elevated in human head and neck squamous cell carcinoma (HNSCC) and iASPP overexpression significantly correlates with tumor malignant progression and poor survival of HNSCC. This study investigated the function of iASPP playing in proliferation and invasion of HNSCC *in vitro*.

Methods HNSCC cell line Tu686 transfected with Lentiviral vector-mediated iASPP-specific shRNA and control shRNA were named the shRNA-iASPP group and shRNA-NC group, respectively. The non-infected Tu686 cells were named the CON group. CCK-8 assay, flow cytometry, transwell invasion assay were performed to detect the effects of iASPP inhibition *in vitro*.

Results Our results demonstrated that the proliferation of shRNA-iASPP cells at the time of 72 h ($F=32.459, P=0.000$), 96 h ($F=51.407, P=0.000$), 120 h ($F=35.125, P=0.000$) post-transfection, was significantly lower than that of shRNA-NC cells and CON cells. The apoptosis ratio of shRNA-iASPP cells was $9.42\% \pm 0.39\%$ ($F=299.490, P=0.000$), which was significantly higher than that of CON cells ($2.80\% \pm 0.42\%$) and shRNA-NC cells ($3.18\% \pm 0.28\%$). The percentage of shRNA-iASPP cells in G0/G1 phase was $74.65\% \pm 1.09\%$ ($F=388.901, P=0.000$), which was strikingly increased, compared with that of CON cells ($55.19\% \pm 1.02\%$) and shRNA-NC cells ($54.62\% \pm 0.88\%$). The number of invading cells was 56 ± 4 in the shRNA-iASPP group ($F=84.965, P=0.000$), which decreased significantly, compared with the CON group (111 ± 3) and the shRNA-NC group (105 ± 8). The survival rate of shRNA-iASPP cells administrated with paclitaxel was highly decreased, compared with CON cells and shRNA-NC cells ($F=634.841, P=0.000$).

Conclusion These results suggest iASPP may play an important role in progression and aggressive behavior of HNSCC and may be an efficient chemotherapeutic target for the treatment of HNSCC.

Received April 11, 2019. Published online July 2, 2019.

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Supported by Beijing Medical Health Public Welfare Foundation (grant no. YWJKJHHKYJJ-B17468 and running period: 2017.09.01-2020.09.01).

HEAD and neck squamous cell carcinoma (HNSCC) is the sixth leading cancer worldwide,^[1] causing a significant morbidity with an incidence of approximately 550 000 cases worldwide annually.^[2] Despite advances in diagnosis and treatment, HNSCC is still a serious threat to human life with a 5-year survival rate of only 50% to 60%.^[3] Similar to all solid tumors, a series of genetic alterations are common in the initiation and progression of HNSCC. Therefore, identifying novel therapeutic targets and biomarkers, and furthermore elucidating their underlying mechanism are efficient ways for prediction, diagnosis and further successful prevention and therapy for HNSCC patients.^[4, 5]

The apoptosis stimulating protein of p53 (ASPP) family, including 3 members, ASPP1, ASPP2, and iASPP, was identified as specific regulators of p53-, p63-, and p73-mediated apoptosis.^[6] iASPP, encoded by PPP1R13L gene, is the evolutionally conserved inhibitory member of the ASPP family. It can specifically inhibit p53-mediated cell apoptosis, and its overexpression confers resistance to ultraviolet radiation and cisplatin-induced apoptosis in cultured cells.^[7] Furthermore, the oncogenic role of iASPP has been shown in many kinds of human cancers, such as breast carcinomas,^[7] acute leukemia,^[8] hepatocellular carcinoma,^[9] ovarian cancer,^[10] gastric cancer,^[11] colorectal cancer,^[12] non-small-cell lung cancer,^[13] prostate cancer,^[14] *et al.* In our previous study, we have demonstrated that iASPP is elevated in human HNSCC and iASPP overexpression significantly correlates with tumor malignant progression and poor survival in HNSCC.^[15]

However, the role of iASPP playing in tumorigenesis and progression of HNSCC is not clear. In this study, firstly we used a lentiviral RNAi system to inhibit the expression of iASPP in HNSCC cell line—Tu686 cells. Then, we investigated the effects of downregulation of iASPP expression on HNSCC cell proliferation, apoptosis, cell cycle, invasion and paclitaxel chemosensitivity *in vitro*.

MATERIALS AND METHODS

Cell culture

HNSCC cell line Tu686 was established from a primary tumor located in the base of tongue. The Tu686 cell line kindly provided by Dr. Zhuo (Georgia) Chen (Emory University Winship Cancer Institute, Atlanta, Georgia) was cultured in a Dulbecco's modified Eagle's

medium (DMEM)/F12 medium (1:1) supplemented with 10% fetal bovine serum (FBS) and antibiotics in a humidified atmosphere with 5% CO₂ at 37°C. Exponentially growing cells were used in the subsequent experiments.

Lentiviral vectors mediated iASPP-specific shRNA transfection and grouping

iASPP-specific shRNA lentiviral particles (sc-72100-V/200 μl, Santa Cruz) are a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19–25 nt (plus hairpin) shRNA designed to knock down iASPP gene expression in human Tu686 cells.

Tu686 cells (2×10^4) were seeded into 24-well plates, and divided into three groups: LV-shRNA-iASPP, LV-shRNA-NC, and non-infected groups (CON). Then the medium containing iASPP shRNA or control shRNA lentiviral particles was added to Tu686 cells of the LV-shRNA-iASPP or LV-shRNA-NC group and incubated for 12 h according to the manufacturer's protocol. The silencing efficiency was analyzed by RT-PCR and western blotting 72 h post-infection.

Quantitative real-time RT-PCR analysis

Quantitative real-time RT-PCR (qPCR) analysis was performed as described in our previous study.^[15] Total RNA was extracted using Trizol reagent (Invitrogen) according to manufacturer's protocol. First-strand cDNA was synthesized with a Revert Aid First Strand cDNA Synthesis Kit (FERMENTAS, USA). qPCR was performed on a MiniOpticon Real-Time PCR System using SYBR green Master Mixture. The primers for iASPP and GADPH amplification were as follows: iASPP (180 bp), 5'-GAAAGCCTGGAACGAGTCTG-3' (forward) and 5'-GC-GCTAGTGAGTTGTCCTT-3' (reverse); GADPH (179 bp), 5'-CGACCACTTTGTCAAGCTCA-3' (forward) and 5'-ACTGAGTGTGGCAGGGACTC-3' (reverse). The mRNA expression levels of iASPP were normalized against GADPH and determined by $2^{-\Delta\Delta CT}$ method.^[16]

Western blotting

Western blotting was performed as previously described.^[17] The same amount of proteins (50 μg) was separated by 10% SDS-PAGE, and the dilution of the antibodies was as follows: anti-iASPP rabbit polyclonal antibody (ab-34898, 1:1500, Abcam), anti-bax rabbit polyclonal antibody (1:1000, Cell Signaling), and anti-bcl-2 rabbit polyclonal antibody (1:1000, Proteintech

Group).

Cell proliferation assay

Cell Counting Kit-8 (CCK8, Beyotime, China) assay was carried out to draw cell growth curves according to manufacturer's protocol. Briefly, cells were seeded into a 96-well plate at a density of 3.0×10^3 /well, cultured with medium containing 10% FBS in a humidified atmosphere with 5% CO₂ at 37°C and exposed to fresh media every other day. The absorbance at 450 nm of the cells in each group was measured at 24, 48, 72, 96 and 120 h post-transfection, and then cell growth curve was generated.

Flow cytometry

After incubated with serum-free medium for 24 h, the cells were washed twice with PBS at 4°C and resuspended to a concentration of 1×10^6 /ml. Subsequently, the cells were incubated with Annexin V-FITC and propidium iodide for 30 min. Fluorescence-activated cell sorting analysis was performed with a Cytomics™ FC500 instrument (Beckman Coulter, USA). Cell apoptosis and cell cycle distribution were measured.

Transwell invasion assay

Transwell invasion assay was performed as previously described.^[18] A 24-well transfected chamber (Costar, Cambridge, MA, USA) was used to evaluate the invasiveness of the transfected cells according to manufacturer's instructions. In brief, 2×10^4 cells in 100 μl serum-free medium were seeded into the upper chamber of the transwell that had been pre-coated with matrigel overnight. The lower chamber was filled with 600 μl DMEM/F12 containing 10% FBS to induce chemotaxis. After 48 h of incubation, the cells were fixed by methanol and stained with crystal violet. The invading cells that were on the lower surface of the filter membrane, were counted under a microscope at $\times 200$ magnification from at least five random fields. Each experiment was repeated three times.

Analysis of sensitivity of Tu686 cells to chemotherapeutics paclitaxel

Cells seeded in a 96-well plate were cultured overnight, and incubated with different concentrations of paclitaxel (0.01, 0.1, 1, 10, 10^2 , 10^3 , 10^4 nmol/L) for 48 h in a CO₂ incubator. The cell viability was assessed by CCK8 assay. The cell survival rate was calculated according to the measured OD450 value: cell survival

rate = (experimental group OD450 – blank OD450) / (control group OD450 – blank OD450) \times 100%. And the half maximal (50 percent) inhibitory concentration (IC50 value) of paclitaxel was calculated. Apoptosis and cell cycle distribution were assayed with flow cytometry.

Statistical analysis

All statistical analyses were performed with SPSS 19.0 software. Quantitative data in this study were presented as mean \pm SD. Two-tailed Student's *t*-test and one way ANOVA followed by Student-Newman-Keuls's post hoc test were used to compare statistical differences between groups. *P* < 0.05 was considered statistically significant.

RESULTS

iASPP shRNA lentiviral particles efficiently silence the expression of iASPP in HNSCC cell line Tu686

qPCR analysis and Western blotting showed lentivirus-delivered iASPP shRNA effectively infected Tu686 cells, and inhibited iASPP mRNA expression more than 80% (Figure 1A) and iASPP protein expression over 70% (Figure 1B, 1C).

iASPP knockdown inhibits the cellular proliferation *in vitro*

To investigate the function of iASPP on cellular proliferation of HNSCC cells *in vitro*, a CCK-8 assay was used. The results showed the proliferation of Tu686 cells was markedly inhibited after knockdown of iASPP gene expression. The OD450 value of shRNA-iASPP cells at the time of 72 h ($F=32.459$, $P=0.000$), 96 h ($F=51.407$, $P=0.000$), 120 h ($F=35.125$, $P=0.000$) post infection, was significantly lower than that of shRNA-NC cells and CON cells. (Figure 2).

iASPP knockdown induces apoptosis and cell cycle arrest at G0/G1 phase

To clarify the possible mechanisms involved in iASPP-mediated cell proliferation, flow cytometry was applied to detect apoptosis and cell cycle. The results showed that the apoptosis ratio of shRNA-iASPP cells was $9.42\% \pm 0.39\%$, which was significantly higher than that of CON cells ($2.80\% \pm 0.42\%$) and shRNA-NC cells ($3.18\% \pm 0.28\%$, $F=299.490$, $P=0.000$; Figure 3).

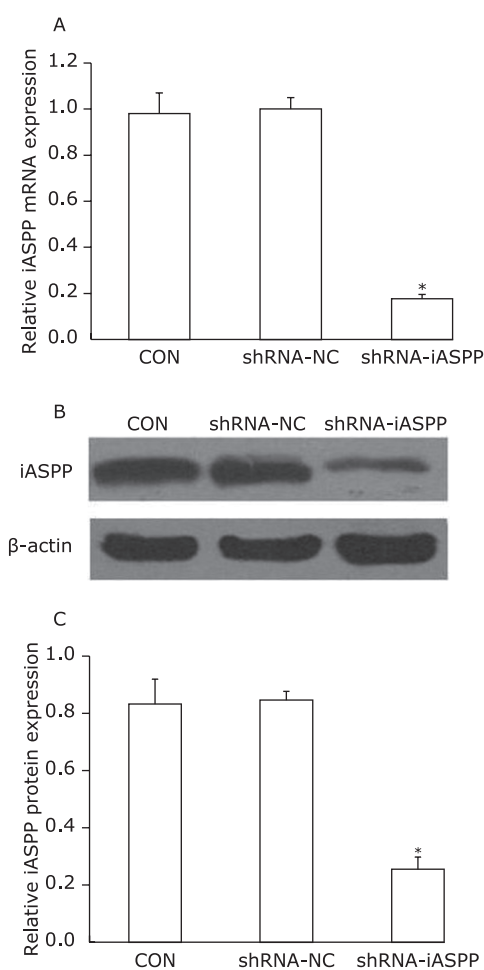


Figure 1. Knockdown of iASPP expression by lentiviral vector-mediated shRNA interference in Tu686 cell lines.

CON: non-infected cells; shRNA-NC: cells transfected with control shRNA lentiviral vector; shRNA-iASPP: cells transfected with iASPP lentiviral vector.

A. Relative iASPP mRNA expression was detected by qPCR. GAPDH was used as an internal control ($^*P = 0.000$, compared with the shRNA-NC group, Student's *t*-test, $n=3$). B. Decreased protein expression of iASPP determined by Western blotting. C. Relative expression levels of iASPP protein ($^*P = 0.000$, compared with the shRNA-NC group, Student's *t*-test, $n=3$).

The percentage of shRNA-iASPP cells at G0/G1 phase was $74.65\% \pm 1.09\%$ ($F=388.901$, $P=0.000$), which was strikingly increased, compared with that of CON cells ($55.19\% \pm 1.02\%$) and shRNA-NC cells ($54.62\% \pm 0.88\%$). Whereas the percentage of shRNA-iASPP cells at S phase was $17.54\% \pm 1.21\%$ ($F=176.759$, $P=0.000$), which decreased significantly, compared with CON cells ($32.56\% \pm 0.98\%$) and shRNA-NC cells ($32.50\% \pm 1.17\%$). The percentage of

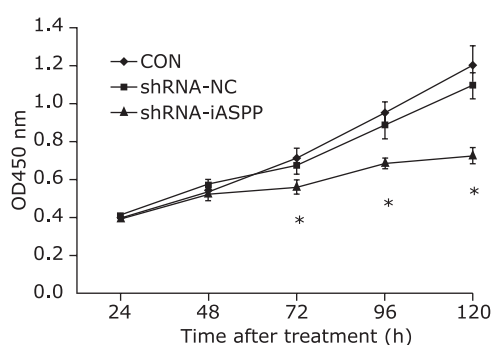


Figure 2. The effect of iASPP knockdown on the proliferation of Tu686 cells. Cell proliferation was measured by the CCK-8 assay every 24 hour for 5 days. Results were expressed as means of three independent experiments \pm SD ($^*P < 0.01$, compared with shRNA-NC cells and CON cells).

shRNA-iASPP cells at G2/M phase was $7.81\% \pm 0.76\%$ ($F=59.999$, $P=0.000$), which reduced prominently, compared with CON cells ($12.24\% \pm 0.68\%$) and shRNA-NC cells ($12.87\% \pm 0.31\%$, **Figure 4**).

The potential effects of downregulation of iASPP expression on some apoptosis-related proteins such as bax, bcl-2 were also evaluated by western blotting. The results demonstrated that downregulation of iASPP expression obviously inhibited the expression of anti-apoptotic protein bcl-2, but significantly increased the expression of pro-apoptotic protein bax (**Figure 5**).

Silencing iASPP expression inhibits cell invasion *in vitro*

To elucidate the effect of iASPP on HNSCC cell invasion, transwell invasion assay was carried out. The result demonstrated invasiveness of the Tu686 cells decreased significantly in shRNA-iASPP cells, compared with CON cells and shRNA-NC cells (**Figure 6**). The number of invading cells was 56 ± 4 in the shRNA-iASPP group ($F=84.965$, $P=0.000$), which decreased significantly, compared with the CON group (111 ± 3) and the shRNA-NC group (105 ± 8). These results suggests that iASPP plays an important role in mediating cell invasion of HNSCC, which is a crucial influence of progression and aggressive behavior of HNSCC.

Down-regulated iASPP expression sensitizes Tu686 cells to paclitaxel *in vitro*

To evaluate the effect of knockdown of iASPP expression on chemosensitivity of HNSCC Tu686 cells to paclitaxel, different concentrations of paclitaxel were

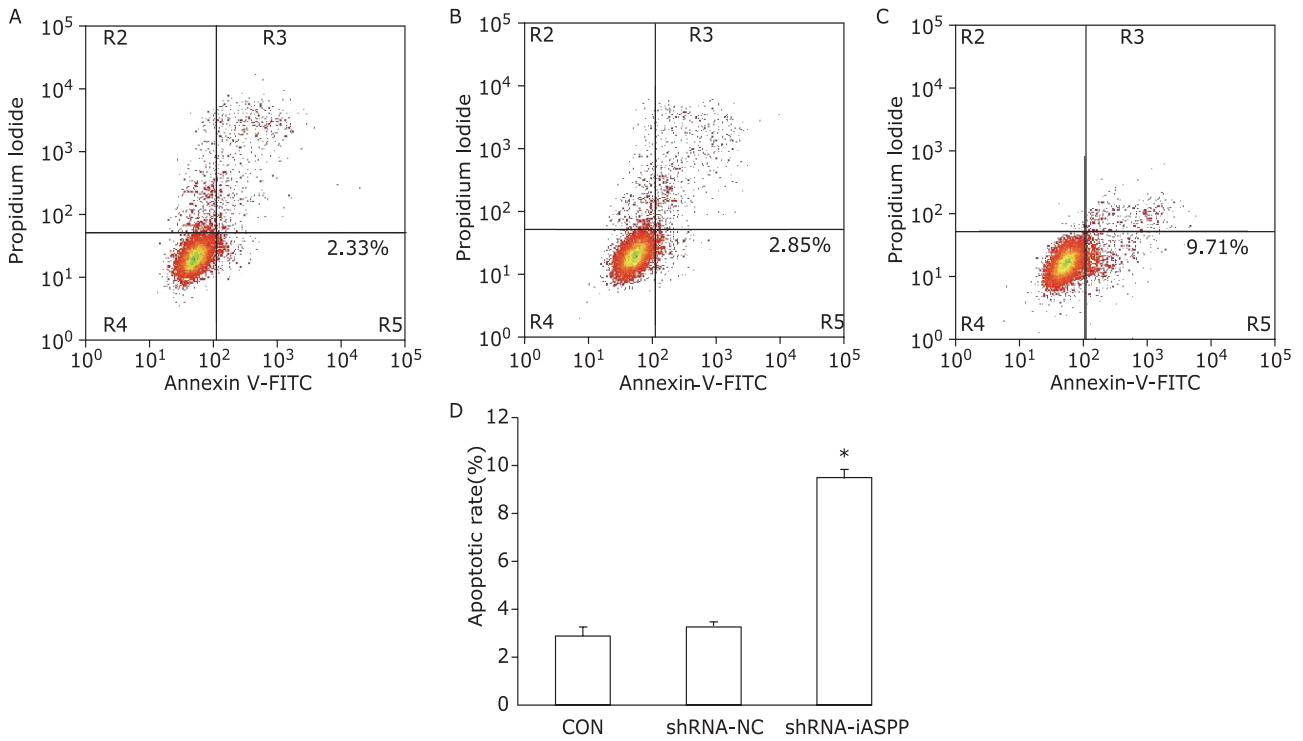


Figure 3. The effect of knockdown of iASPP on cell apoptosis of Tu686 cells was analyzed by flow cytometry. Representative histograms showing apoptosis of CON (A), shRNA-NC (B) and shRNA-iASPP cells (C). The apoptosis ratio of shRNA-iASPP cell line was significantly higher than that of CON cell line and shRNA-NC cell line (D, * $P = 0.000$). Results of quantitative analysis represent the mean \pm SD of three independent experiments.

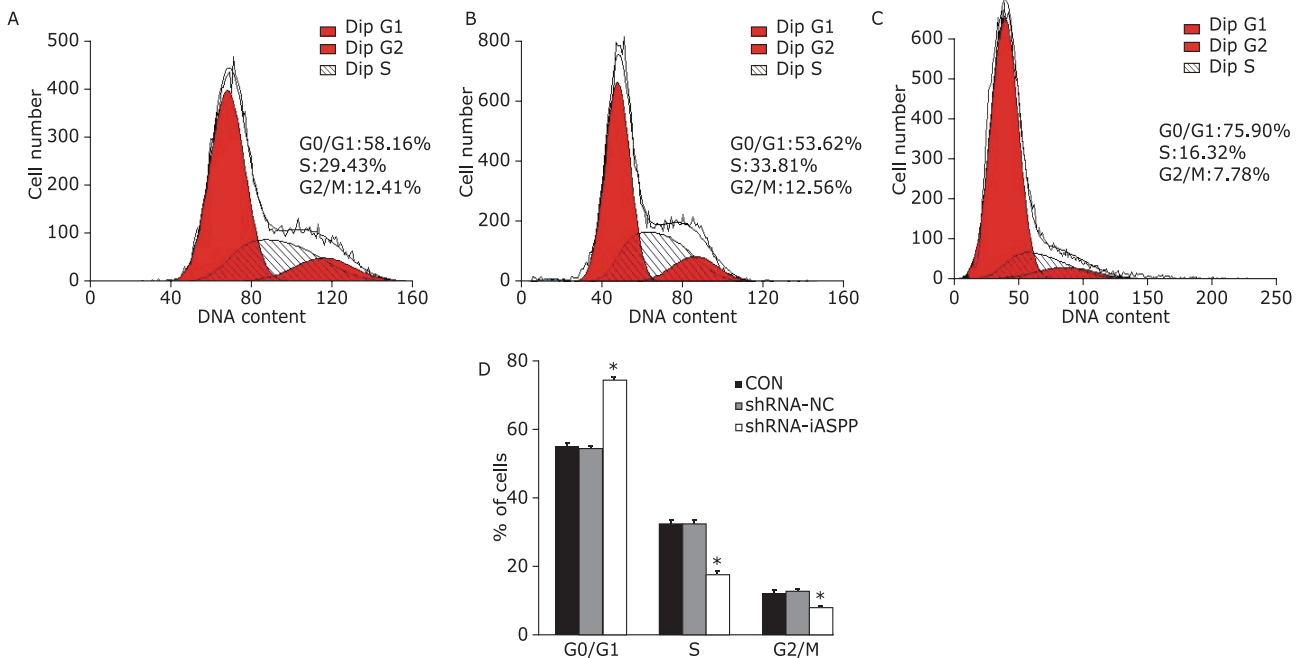


Figure 4. The effect of knockdown of iASPP on cell cycle distribution of Tu686 cells was analyzed by flow cytometry. Representative histograms showing cell cycle distribution of CON (A), shRNA-NC (B) and shRNA-iASPP cells (C). The percentage of shRNA-iASPP cells in G0/G1 phase was strikingly increased (* $P=0.000$), whereas the percentage of cells in S phase and G2/M phase decreased significantly (D) (both * $P=0.000$). Results of quantitative analysis represent the mean \pm SD of three independent experiments.

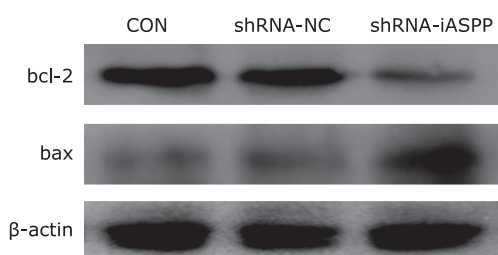


Figure 5. The effect of knockdown of iASPP expression on the expression of apoptosis-related proteins in Tu686 cells was analyzed by western blotting.

As shown above, downregulation of iASPP expression obviously inhibited the expression of anti-apoptotic protein bcl-2, but significantly increased pro-apoptotic protein bax expression.

employed to treat CON cells, shRNA-NC cells and shRNA-iASPP cells for 48 h.

The survival rate of shRNA-iASPP cells exposed to paclitaxel was highly decreased, compared with CON cells and shRNA-NC cells ($F=634.841$, $P=0.000$; **Figure 7**). Calculated by SPSS 19.0, IC₅₀ value of paclitaxel for shRNA-iASPP cells, CON cells and shRNA-NC cells was 3.84 ± 0.28 , 47.46 ± 2.12 and 50.93 ± 2.27 nmol/L, respectively. These results demonstrated the chemosensitivity of shRNA-iASPP cells to paclitaxel was highly increased, compared with CON cells and shRNA-NC cells.

After treatment with IC₃₀ of paclitaxel, flow cytometry showed the apoptosis ratio of shRNA-iASPP cells was strikingly increased, compared with CON cells and shRNA-NC cells ($18.27\% \pm 1.13\%$ vs. $7.31\% \pm 0.33\%$, $8.20\% \pm 0.34\%$, $F=220.453$, $P=0.000$; **Figure 8**). Flow cytometry also demonstrated the percentage of shRNA-iASPP cells at G₂/M phase was obviously increased, compared with CON cells and shRNA-NC cells ($28.68\% \pm 0.76\%$ vs. $19.21\% \pm 0.89\%$, $17.99\% \pm$

0.13% ; $F=219.075$, $P=0.000$), whereas the percentage of cells at S phase decreased significantly, compared with CON cells and shRNA-NC cells ($15.11\% \pm 1.09\%$ vs. $26.38\% \pm 2.04\%$, $26.58\% \pm 0.67\%$, $F=66.760$, $P=0.000$). For G₀/G₁ phase, no obvious difference was found among the three groups ($56.21\% \pm 1.82\%$ vs. $54.41\% \pm 1.15\%$, $55.42\% \pm 0.56\%$, $F=1.479$, $P=0.300$; **Figure 9**). These data demonstrated inhibiting iASPP expression can significantly promote cell apoptosis and induce cell cycle arrest, which can strengthen chemosensitivity of HNSCC cell line to paclitaxel. It suggests that iASPP may be a novel and efficient chemotherapeutic target for HNSCC treatment.

DISCUSSION

We investigated the function of iASPP associated with tumorigenesis, invasiveness and chemosensitivity of HNSCC *in vitro*. The results have clearly demonstrated that silencing iASPP gene expression could promote apoptosis, arrest cell cycle at G₀/G₁ phase, inhibit cell proliferation and invasion of *in vitro* HNSCC cell line, and strengthen chemosensitivity of HNSCC cell line to paclitaxel. All these findings suggest that iASPP could play an important role in progressive and aggressive behavior of HNSCC and might be a novel and efficient chemotherapeutic target for HNSCC treatment.

Several studies have revealed that iASPP knockdown inhibits cellular proliferation and induces cell apoptosis in several cancers such as colorectal cancer,^[19] spinal chordoma,^[20] leukemia,^[21] cervical cancer,^[22] glioblastoma,^[23] gastric cancer,^[24] *et al.*, which were in consistent with the results of our study. Our result showed downregulation of iASPP expression increased bcl-2 expression and decreased bax expression, which were similar to those found in spinal

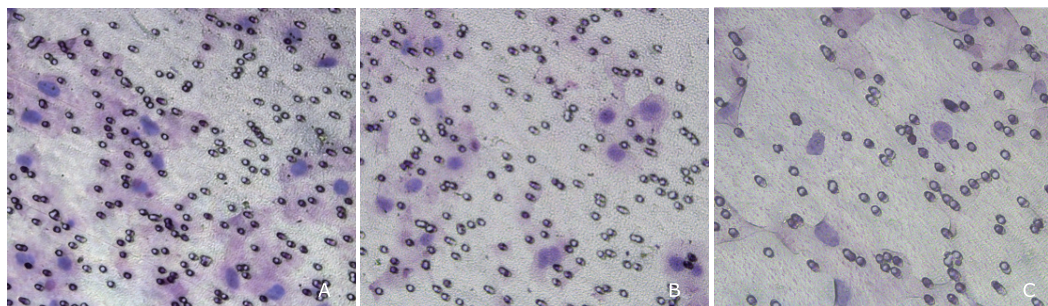


Figure 6. Downregulation of the expression of iASPP inhibited the invasion of Tu686 *in vitro*. Matrigel invasiveness assay in transwell culture chamber showed invasiveness decreased significantly in the shRNA-iASPP group (C), compared with the CON group (A) and shRNA-NC group (B).

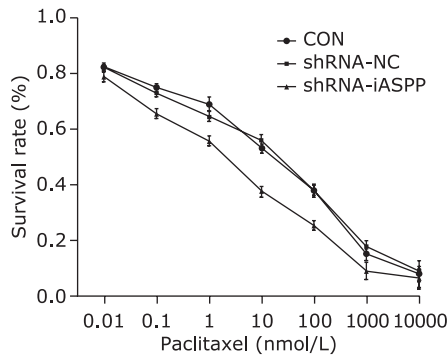


Figure 7. Knockdown of iASPP expression can strengthen chemosensitivity of HNSCC Tu686 cell line to paclitaxel. CON, shRNA-NC and shRNA-iASPP cells were treated with increasing concentrations of paclitaxel for 48 h, and the cell survival rate was assessed by CCK-8 assays. Results represent the mean \pm SD of three independent experiments.

chordoma.^[20] The above results indicated that iASPP can inhibit cell apoptosis by regulating apoptosis-related proteins.

In our previous study, we have found overexpression of iASPP was significantly correlated with lymph node metastasis.^[15] This study has verified silencing iASPP expression can inhibit invasion of the Tu686 cells

in vitro. These results revealed iASPP might associate with lymph node metastasis of HNSCC, which is crucial for invasion and metastasis of HNSCC. Consistently, Morris *et al.*^[25] reported that the increased iASPP was associated with tumor invasive growth, metastatic disease, and cancer-related mortality. They revealed that iASPP was enriched in highly metastatic prostate cancer cells compared to primary cells, and iASPP-overexpressing cells distributed at the invasive leading edge mostly. Ma *et al.*^[20] regarded that the elevated iASPP expression had significant association with tumor invasion. It has also demonstrated that downregulated iASPP expression could remarkably weaken the proliferative and invasive properties of the pancreatic adenocarcinoma cells and the central nervous system lymphoma cells.^[26, 27] Previous studies showed some microRNAs could regulate iASPP expression and mediate malignant progression including cellular invasion of many tumor cells, such as glioblastoma, prostate cancer and colorectal cancer.^[28-30] Zhao *et al.*^[31] revealed the interaction of lncRNA-microRNA-iASPP and identified the H19/miR-140/iASPP axis may participate in regulating proliferation and invasion of glioma

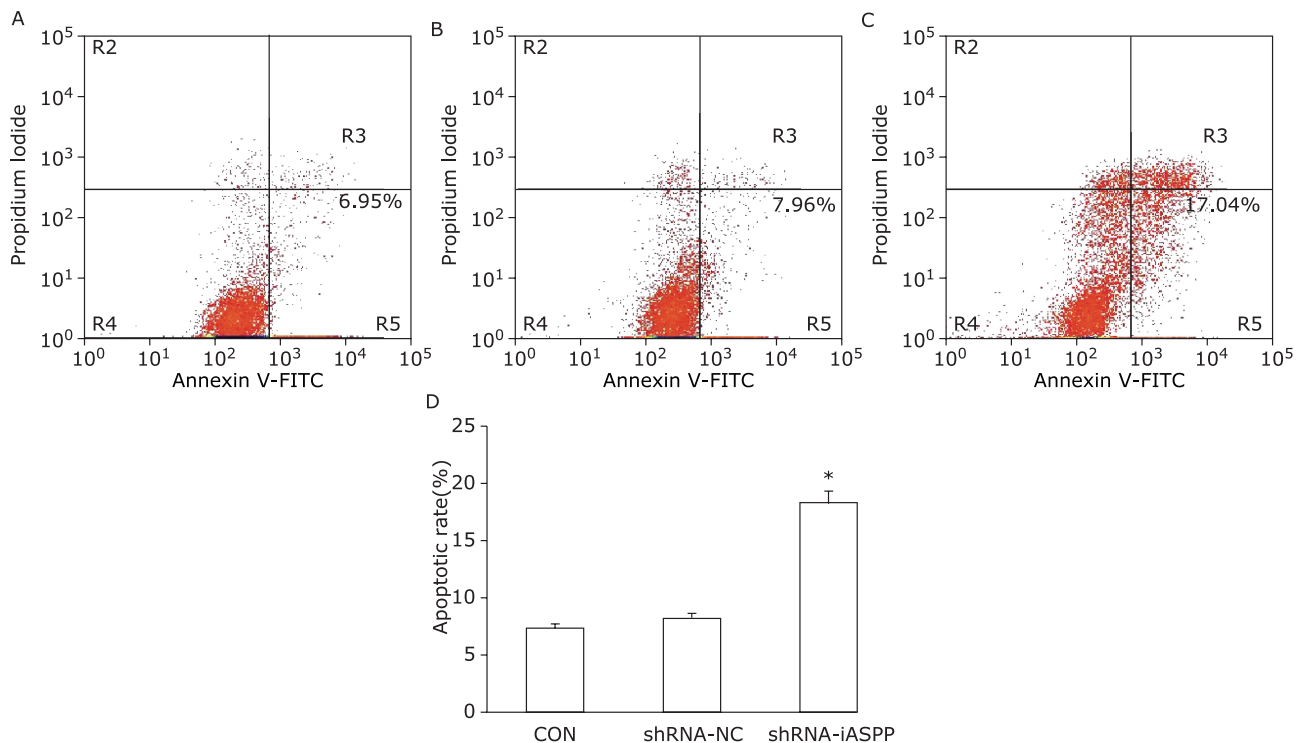


Figure 8. The effect of knockdown of iASPP expression on cell apoptosis of Tu686 cells treated with paclitaxel for 24 hours was analyzed by flow cytometry. Representative histograms showing apoptosis of CON (A), shRNA-NC (B) and shRNA-iASPP cells (C). The apoptosis ratio of shRNA-iASPP cells was significantly higher than that of CON cells and shRNA-NC cells (D) (* $P=0.000$). Results of graphic D represent the mean \pm SD of three independent experiments.

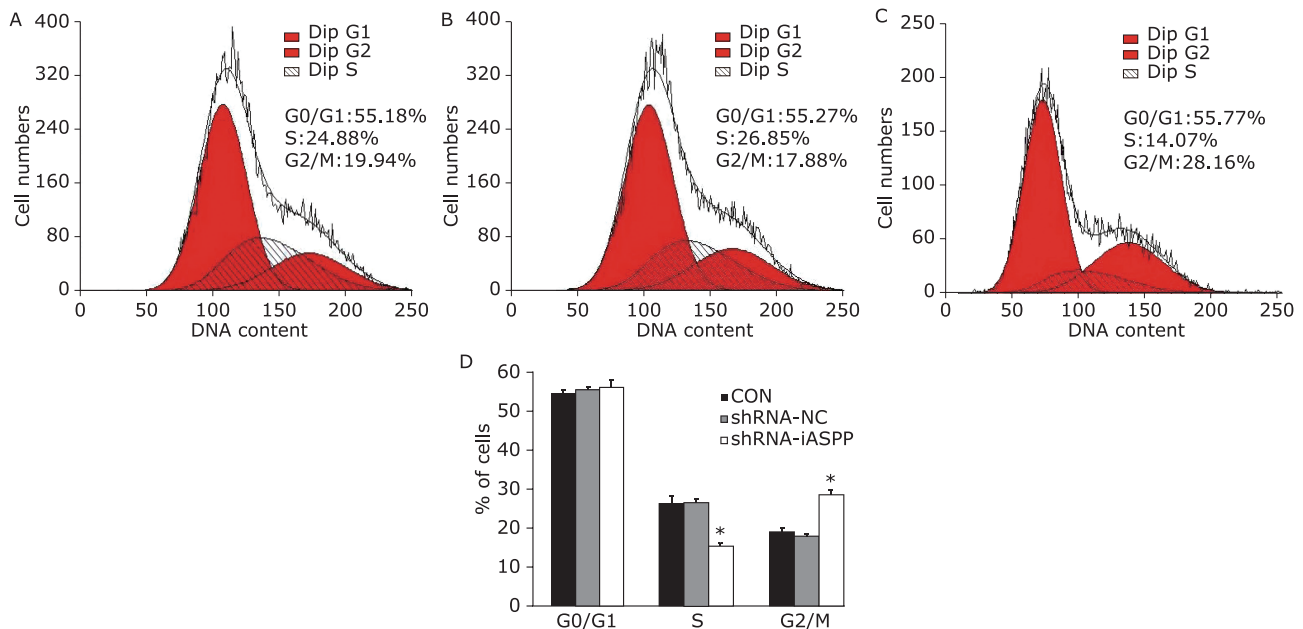


Figure 9. The effect of knockdown of iASPP expression on cell cycle distribution of Tu686 cells treated with paclitaxel for 24 hours was analyzed by flow cytometry. Representative histograms showing cell cycle distribution of CON (A), shRNA-NC (B) and shRNA-iASPP cells (C). The percentage of shRNA-iASPP cells in G2/M phase was strikingly increased ($*P=0.000$), and the percentage of cells in S phase decreased significantly ($*P=0.000$). Results of graphic D represent the mean \pm SD of three independent experiments.

cells. Xiong *et al.*^[32] verified that iASPP can stimulate epithelial-mesenchymal transition by regulating the downstream signaling pathway of miR-20a-FBXL5/BTG3 in cervical adenocarcinoma cells. Liang *et al.*^[27] suggested that miR-184 targeting iASPP could suppress invasion of human central nervous system lymphoma cells through regulating PI3K/AKT signaling pathway. iASPP has been shown to promote oncogenic features *via* interacting with p53 and inhibiting its ability to regulate transcription of target genes and repressing the expression of p53-responsive miR-34a.^[22] All these evidences indicate that the regulatory mechanisms of iASPP on invasion are complicated and diverse. Therefore, further researches are necessary to explore the definite mechanism in future.

Chemotherapy is part of the systemic treatment for HNSCC. Paclitaxel, a tubulin-stabilizing anti-cancer agent, is now widely used to treat HNSCC. Many trials have indicated that a three-drug regimen consisting of taxel (including docetaxel), cisplatin and 5-fluorouracil (TPF) improves outcomes of patients with HNSCC.^[33-35] However, drug-resistance seriously interferes with the effectiveness of chemotherapy. In fact, increased iASPP expression in human malignant cells is mainly associated with their chemoresistance.^[7] Yu *et al.*^[36]

reported the expression of iASPP in gastric carcinoma (GC) patients with cisplatin resistance was significant higher than that in the healthy control group. In addition, higher expression of iASPP was also detected in cisplatin-resistant cancer cell lines. They concluded that iASPP induced cisplatin resistance in GC patients and iASPP might be a novel therapeutic strategy for the treatment of cisplatin-resistant GC. Jiang *et al.*^[10] reported that iASPP overexpression in ovarian cancer cells conferred resistance to paclitaxel by reducing mitotic catastrophe in a p53-independent manner *via* activating separase, whereas silencing iASPP expression enhanced paclitaxel-mediated mitotic catastrophe through inactivating separase. Xiong *et al.*^[32] found that knockdown of iASPP suppressed cervical cancer cell proliferation and sensitized cervical cancer cells to cisplatin *in vivo*. Jia *et al.*^[37] observed that in response to cell damage stimuli, iASPP cells can protect hematopoietic cells against apoptosis; in the meantime these apoptosis-resistant cells would have more mutation accumulation. Cao *et al.*^[38] found that iASPP was highly elevated in human cervical cancer, and overexpression of nuclear iASPP was correlated with poor prognosis and chemoresistance/radioresistance, suggesting that iASPP might serve as a novel potential

prognostic marker and therapeutic target for cervical cancer. Furthermore, knockdown of endogenous iASPP caused a significant increase of p53-mediated apoptosis induced by chemotherapeutics in human malignances.^[7, 39] In this study, we confirmed that suppression of iASPP expression can significantly promote cell apoptosis and induce cell cycle arrest, which can strengthen chemosensitivity of HNSCC cell line to paclitaxel. All these evidences suggest that iASPP may be a novel and efficient chemotherapeutic target for HNSCC treatment.

In conclusion, knockdown of iASPP expression inhibited proliferation and invasion, and strengthen chemosensitivity of HNSCC to paclitaxel *in vitro*. It suggests iASPP might play an important role in progression and aggression of HNSCC and serve as a potential therapeutic target for HNSCC. Furthermore, the potential mechanisms involved iASPP in the tumorigenesis and progression of HNSCC should be studied in the future.

Conflicts of interest statement

The authors declare no conflicts of interest.

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