Silencing of DYRK1B improves the sensitivity of human cervical carcinoma cells to cis-platinum

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Abstract: Aim: The aim of this study was to explore whether downregulation of DYRK1B (shDYRK1B) could improve cervical cancer cells sensitivity to cisplatin (DDP). Methods: The expression level of DYRK1B in human cervical cancer cell lines was detected by real-time PCR. SiHa cells showed the highest expression of DYRK1B, and were infected with a lentivirus containing small hairpin RNA (shRNA), designed to silence expression of DYRK1B. Expression of DYRK1B, Cdc2, Cyclin B1, and p53 were detected by Western blot. MTT assay was used to detect the sensitivity of SiHa cells to DDP. After treatment with DDP on shDYRK1B cells, PI staining and Annexin V/PI double staining were used to evaluate cell cycle proportion and cell apoptosis rate respectively. Cell scratch assay and transwell assay were employed to evaluate the ability of cell migration and invasion. Results: After downregulation of DYRK1B in SiHa cells, and following treatment with DDP, the apoptosis rate and phosphorylation levels of p53 were significantly increased (P < 0.05, vs shScramble group), and DYRK1B expression as well as IC₅₀ of DDP were decreased in shDYRK1B group (P < 0.05). A total of 69.51% cells were arrested in the G2/M phase with a marked up-regulation of Cyclin B1 and Cdc2 protein levels in shDYRK1B group. Moreover, the ability of cell migration and invasion were greatly abolished in the shDYRK1B group. Conclusion: shDYRK1B inhibits DYRK1B expression and increases the apoptosis rate of DDP-treated cancer cells by upregulating phospho-p53 and improves SiHa cells sensitivity to DDP. Downregulation of DYRK1B also induced G2/M arrest in SiHa cells and reduced the ability of cell migration and invasion. Targeting DYRK1B may provide a new approach for DPP-based cervical cancer chemotherapy.

Keywords: Small hairpin RNA (shRNA), DYRK1B, cervical cancer cells, chemotherapy sensitivity

Introduction

In women, cervical cancer is the third most commonly diagnosed malignant tumor of the reproductive tract worldwide, with approximately 470,000 new cases and 233,000 deaths per year, especially for women aged 30-55 years [1]. China accounts for 29.0% of the 470,000 new cases of cervical cancer each year [2]. Early stages of cervical cancer have no symptoms but as the disease develops, more and more serious symptoms and signs may develop, such as vaginal bleeding, frequent micturition, urgent urination, constipation, and anemia. Early stages of cervical cancer are mainly cured by removing or destroying the pre-cancerous or cancerous tissue by surgery or radiotherapy, supplemented with chemotherapy in some cases [3, 4]. Improvement of chemotherapy sensitivity of cervical cancer has become an important research trend due to the poor chemotherapy sensitivity of some drugs like cisplatin (DDP), whose sensitivity only occupied 20-30% [5].

Mirk (minibrain-related kinase) is a member of the minibrain/dyrk family of related serine/threonine kinases which is also known as the dual specificity tyrosine-phosphorylation-regulated kinase 1B (Dyrk1B). Mirk/Dyrk1B has the ability to auto-phosphorylate on tyrosine activating itself and then phosphorylate other substrates...
on serine and threonine. Therefore, it has been categorized as a dual function kinase. DYRK1B expression is low or negative expression in most tissues but is elevated in many tumors [6, 7]. Interestingly, knocking down DYRK1B induced apoptosis and increased sensitivity of human cancer cells to therapeutic agents [8, 9]. It was thought to play a role in maintaining the G0 quiescent state by phosphorylating and stabilizing p27 and destabilizing cyclin D1 [10], the quiescent cancer cells depleted of Mirk/DYRK1B went out of G0 phase and entered into the cell cycle. This may enhance the killing effects caused by chemotherapeutic drugs or radiation, while having less effect on normal tissues in which Mirk/DYRK1B levels are quite low [11]. The presence of drug-resistant, higher proportions of quiescent cancer cells with high clonogenic capacity and tumorigenicity, is known to increase recurrence of human cancer and shorten patient survival rate. In previous studies, to explore the clinical significance of Mirk/DYRK1B in cervical tissue, we also detected the expression levels of Mirk/DYRK1B in normal tissues, para-carcinoma tissues, and cervical carcinoma tissues by reverse-transcriptase polymerase chain reaction (RT-PCR) and immunohistochemical (IHC) and found that the expression level of DYRK1B in cervical carcinoma tissue was significantly higher than that in para-carcinoma tissue and normal cervical tissue ($\chi^2 = 94.0, P < 0.001$) [13]. In our present study, we use lentivirus to knockdown expression of DYRK1B and evaluate the sensitivity of cervical cancer cells to DDP and to explore possible mechanisms.

Materials and methods

Material reagents

Human cervical cancer Hela, c33a, and SiHa cells were all purchased from China Center for Type Culture Collection (CCTCC). DYRK1B shRNA and scramble shRNA were synthesized by Sangon Biotech (Shanghai) Co., Ltd. DDP was obtained from Shandong Qilu Pharmaceutical Factory (purity 99.9%); Kits for RNA reverse-transcription and PCR were purchased from TaKaRa; Thiazolyl blue (MTT) was obtained from Sigma; Annexin V-FITC/PI double dye kits came from Invitrogen; Cell culture medium including RPMI1640, DMEM medium and fetal bovine serum (FBS) were purchased from Life technology. BCA Protein Assay Kit was purchased from Beyotime Institute of Biotechnology; DYRK1B antibody (#2703S), p53 antibody (#9282), phospho-p53 (Ser20) antibody (#9287), cdc2 antibody (#77055), Cyclin B1 antibody (#4138), and β-actin antibody (#3700s) were all purchased form Cell Signaling Technology, Inc.

Cell culture

The cervical cancer cell line SiHa and c33a cells were cultured in DMEM medium containing 10% FBS and ampicillin-streptomycin (100 U/ml each). HeLa cells were cultured in RPMI1640 medium, containing 10% FBS and ampicillin-streptomycin (100 U/ml each). Cells were placed in a humidity incubator with temperature of 37°C and 5% CO$_2$.

Construction of lentiviral vector and establishment of silencing DYRK1B cell lines

According to the full-length DYRK1B mRNA sequence and the shRNA primer design principle, the specific shRNA sequence was designed by using shRNA on-line design software (http://www.ambion.com) and synthesized by Sangon Biotech, Co., Ltd. 3 pairs of shRNA target DYRK1B were designed and listed as follows:

| shDYRK1B-1F | CCAGGACCGCTGAGAAGAGATTATTCGAGAATATCGGGTTCTGTAAGCGGTTTTTTG |
| shDYRK1B-1R | AATTCAAAAAACCGCTACAGCAACCGATTTCGAGAATATCGGGTTCTGTAAGCGGTTTTTTG |
| shDYRK1B-2F | CCAGGACGACATCGCTAGCTGTCAGCCGAATTTCGAGAATATCGGGTTCTGTAAGCGGTTTTTTG |
| shDYRK1B-2R | AATTCAAAAAACGAAATTGACTCGCTCATTGCTCGAGCAATGAGCGAGTCAATTTCGT |
| shDYRK1B-3F | CCAGGACCGAGATGAGAACATCGCTCATTGCTCGAGCAATGAGCGAGTCAATTTCGT |
| shDYRK1B-3R | AATTCAAAAAACGAAATTGACTCGCTCATTGCTCGAGCAATGAGCGAGTCAATTTCGT |

shRNA without homology to DYRK1B gene and other genes were selected as the negative control (shScramble), and its sequence was 5'-CCG GAC GGA GAT GAA GTA TAT ACT CGA GTA TAT AGT ACT TCA TCT CGG TTT TTT TTG G-3'. Double stranded DNA fragments were formed by annealing shDYRK1B-1F and shDYRK1B-1R, so as the other shRNA pairs, and linked to pLKO.1-TRC vector (includes GFP encoding sequence) digested by different enzymes. The ligated plasmid was transformed into E.coli DH5α competent cells for plasmid amplifica-
shDYRK1B improves DDP sensitivity in cervical cancer

For the lentivirus packaging, 24 hours before the transfection, 293T cells were divided into three groups, including a shRNA group, a scramble shRNA group, and a normal control group. At 1 hour before transfection, the cell culture medium was replaced with opti-MEM. After combination of shDYRK1B or shScramble plasmid with the packaging plasmid, Lipo-fectamine™ 2000 was mixed and incubated at room temperature for 20 minutes, and then the total mixture was added to 293T cells. After incubation at 37°C for 6 hours, the medium was replaced with complete medium contained 10% FBS. Then 48 hours later, expression of GFP fluorescence could be observed under fluorescent microscope. Supernatant containing lentivirus particles was collected and filtered through a 0.45 μm syringe filter to infect the cervical cancer cells. Polybrene was used to enhance the infection efficiency, and cell lysate was also collected to evaluate the knock down efficiency.

Real-time PCR

To detect DYRK1B mRNA and p53 mRNA expression in SiHa cells, cells were collected. Total cell RNA was extracted with Trizol (method according to the instruction). The cDNA was synthesized at 42°C for 60 minutes by MMLV reverse transcriptase. Then the real-time PCR was performed with the SYBR Green Master Mix (Applied Biosystems) using the cDNA as the template. The PCR conditions were as follows: a pre-denaturation at 95°C for 1 min followed by 37 cycles of denaturing at 95°C for 15 seconds, annealing at 60°C for 15 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C for 8 minutes. The primer used in real-time PCR listed as follows, DYRK1B: Forward: 5'-CAC ACG CAG GTA TTG CCT GAT-3', reverse: 5'-GTC CAC AGA GAG CTT ACG CA-3'; TP53: Forward: 5'-CAG CAC ATG ACG GAG GTT GT-3', Reverse: 5'-TCA TCC AAA TAC TCC ACA CGC-3'; GAPDH: Forward 5'-ACA ACT TTG TGG TGG AAG G-3' and Reverse 5'-GCC ATC ACG CCA CAG TTT C-3'. Triple duplicates were tested for each group, and this experiment was repeated for three times. Relative expression of targets gene was calculated using GAPDH as internal control.

MTT assay

SiHa cells in logarithmic growth phase were inoculated into 96-well plates (3 × 10^4 cells per well). Three replicates were set for each group and non-DDP-treated group was taken as control. Then 24 hours later, cells were added with different concentrations of DDP respectively. Another 48 hours later, 20 µl MTT (5 mg/ml) was added for 4 hours co-incubation at 37°C. After adding 200 µl DMSO and vibrating for 10 minutes, absorbance (A) value at 560 nm wavelengths was measured by microplate spectrophotometer to evaluate the cell inhibition rate of DDP at different concentrations. The cell growth inhibition rate = (1-OD_{DDP-treated}/OD_{blank}) × 100%. IC_{50} of DDP was calculated according to the correlation between DDP concentration and inhibition rate. These six groups were the control group, the shDYRK1B group, the shScramble group, the control + DDP group, the shDYRK1B + DDP group, and the shScramble + DDP group.

Flow cytometric analysis

For cell cycle analysis, SiHa cells infected with DYRK1B shRNA, shScramble, and empty vector were incubated in a 24-well plate (1 × 10^4 cells/well) and treated with DDP. After 24 hour co-incubation, cells were collected and fixed in cold 70% ethanol overnight at 4°C, followed by PI (1 mg/ml) staining in the presence of 1% RNase A for 30 minutes at 37°C in the dark. Flow-cytometry (BD FACS Calibur, BD, USA) was employed to analyse the proportion of cells in the sub-G1, G0/G1, S and G2/M phases. At least 15,000 cells were harvested for each analysis, and the experiments were performed in triplicate.

For apoptosis detection, SiHa cells in logarithmic growth phase were inoculated in 6-well plates (1 × 10^5 cells per well), six groups with three replicates were set. Cells were digested with trypsin, centrifuged at 1000 rpm for 3 minutes, and then washed three times with PBS. The collected cells were resuspended with 400 µl binding buffer, mixed with 5 µl Annexin V-FITC, and incubated for 10 minutes at room temperature. After adding 5 µl PI, the mixture was incubated at 4°C in the dark for 30 minutes. The apoptosis rate was analyzed using flow cytometry. For each sample, 10000 fluorescent signals were collected for subsequent
shDYRK1B improves DDP sensitivity in cervical cancer

statistical analysis. Apoptosis rate = (apoptotic cells/total cells) × 100%.

siRNA interference

shDYRK1B and shScramble cells (0.4 × 10^6/well) were transfected in 6-well plates with 5 μl of 20 μM siRNA duplexes for TP53 and vehicle control (Life technology, Cat No. 4390824) using Lipo2000 according to the manufacturer’s instructions (Invitrogen). At 24 hours after transfection, cells were treated with 5 μM cisplatin for 48 hours, then cells were harvested and the percentage of apoptotic cells was determined by Annexin V/PI double staining on the flow cytometry as described above.

Western blot

The SiHa cells infected with shDYRK1B, shScramble and empty vector were treated with DDP for 24 hours. Then the cells were collected and lysed in ice-cold RIPA buffer containing a protease inhibitor mixture (Roche). The cell proteins were determined by BCA Protein Assay Kit and electrophoresed on 10% SDS polyacrylamide gel, and then transferred to a PVDF membrane by constant current for 1.5 h. After blocking with 5% skim milk for 1 hour, the membrane was incubated with appropriate primary antibodies overnight at 4°C. The next day, membrane was incubated in the milk with HRP-labeled secondary antibodies at room temperature for 1 hour. The blotting bands were identified by enhanced chemiluminescence (ECL) kits according to the manufacturer’s recommendation.

Scratch assay

A scratch assay was used to assess SiHa cells migration ability after DYRK1B stably silenced, as previously described [13]. SiHa cells were seeded in a six-well plate and grown to about 100% confluence. At time point 0, a pipette tip was used to make a plus-shaped scratch on the bottom of each well. One representative image was taken for each scratch. At 60 hours post scratch, one representative image was taken again for each scratch at approximately the same locations as the first image.

Transwell assay

For cell invasion determined, SiHa cells were digested and re-suspended to 5 × 10^5 cells/ml with RPMI1640 without serum, and then 100 μl this cell suspension was placed into each transwell chamber (8 μm pore size), and on the lower chamber 600 μl RPMI1640 was added with 10% FBS, and three replicates were set for each group. After 12 hours of cultivation, the cells did not go through membrane were eliminated, while the remaining cells at another side of the transwell membrane were fixed with anhydrous ethanol and stained with 0.1% crystal violet. The cells were photo-
shDYRK1B improves DDP sensitivity in cervical cancer

**Results**

**shRNA transfection and DYRK1B mRNA expression in SiHa cells**

The relative RNA expression of DYRK1B in three cervical cell lines was detected by real-time PCR with GAPDH as the internal control, including SiHa (4.8±0.6), HeLa (3.8±0.5), and c33a (2.9±0.3). SiHa was found to have sufficient expression of DYRK1B, and was used for subsequent experiments. Since the plasmids contain sequences for GFP expression, the cells infected with lentivirus containing specific shRNA for DYRK1B had green fluorescence under a fluorescence microscope, as shown in Figure 1A. The SiHa cells infected with shDYRK1B exhibited a significant green fluorescence at 48 hours post-infection, suggesting a desirable infection efficiency, which meant that most SiHa cells were infected with the lentivirus. After infection, cells protein was collected and Western blot was performed to evaluate the downregulation efficiency. It was found that the shRNA-3 had the maximal downregulation efficiency of DYRK1B (data not shown), so the shRNA-3 infected SiHa Cell lines with DYRK1B were stably silenced and were used for the subsequent experiments. As shown in Figure 1B and 1C, when compared with shScramble control and a normal control group, DYRK1B mRNA and protein expression were significantly down-regulated in the shRNA-3 group with silencing efficiency of about 52% (P < 0.05). However, there was no statistical significance between the shScramble group and the normal control group (P > 0.05).

**Effect of shDYRK1B on the cell viability and sensitivity of SiHa cells to DDP**

As shown in Figure 2, after 48 hour treatment with 5 μM DDP, the apoptosis rate of SiHa cells was significantly increased (Figure 2A), while the IC_{50} value of DDP against SiHa cells declined in the shDYRK1B group when compared with the shScramble + DDP group and/or the control + DDP groups (P < 0.05), while there was no statistical significance between the shScramble + DDP group and the control + DDP group (P > 0.05).

**Effect of shDYRK1B on cell cycle arrest in DDP-treated SiHa cells**

MTT assay showed that the cell growth of SiHa cells, after downregulation of DYRK1B, was significantly inhibited. Restrained cell proliferation always indicated cell cycle arrest [14], therefore, the effect of shDYRK1B on the cell cycle arrest was detected by flow cytometric analysis. Figure 3 shows that 69.51% of the cells were arrested at G2/M phase and 10.82% of the population in the G0/G1 phase in shDYRK1B group. Figure 3B shows that in the shScramble

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**Figure 2.** Effects of shDYRK1B on the cell viability and IC_{50} of SiHa cells to DDP. A. Apoptosis rate of SiHa cells; B. The IC_{50} value used for evaluating the sensitivity of SiHa cells to DDP was calculated according to the correlation between DDP contents and GIR in MTT assay. Three replicates were set for each group and this experiment was repeated for three times. *P < 0.05 vs normal control group, **P < 0.05 vs the shScramble group.
shDYRK1B improves DDP sensitivity in cervical cancer

and control groups, the population in the G2/M phase of SiHa cells was 25.32% (vs shDYRK1B, \( P < 0.05 \)), and 29.02% (vs shDYRK1B, \( P < 0.05 \)), respectively. These data indicate that downregulated DYRK1B could arrest the cell cycle at the G2/M phase. Then, the changes of the cell cycle regulatory proteins, including cyclin B1 and Cdc2, were detected. Mitotic arrest induced by downregulation of DYRK1B may be associated with a marked up-regulation of cyclin B1 and Cdc2 protein levels. Furthermore, there was a significant difference of cyclin B1 and Cdc2 expression between the shDYRK1B group and the control or shScramble group (\( P < 0.05 \), \( **P < 0.01 \)).

Effect of shDYRK1B on expression and phosphorylation levels of p53

Stable downregulation of DYRK1B gene led to a higher apoptosis rate in SiHa cells after DDP treatment. p53-dependent apoptosis is the crucial factor governing the inhibiting effects of DDP in SiHa cells. Therefore, the enhanced sensitivity of SiHa cells to DDP after shDYRK1B interference was speculated to be associated with p53-dependent apoptosis as shown in Figure 4A-C.

The results from Western blot and qPCR show that expression of p53 mRNA (Figure 4C) and protein were significantly increased in the shRNA interference group after DDP treatment for 24 hours, as shown in Figure 4B. Additionally, the difference between the shDYRK1B group and the shScramble or normal control group exhibited significance (\( P < 0.05 \)). Meanwhile, up-regulated phosphorylation of p53 was also observed in the shRNA interference group (Figure 4B), suggesting that enhanced sensitivity of SiHa cells to DDP may result from increased phosphorylation of p53. Furthermore, siRNA interference technology was used to downregulate p53, as shown in Figure 4D, and the apoptosis rate was abolished in three DPP-treated groups. In contrast, in the shDYRK1B group, downregulation of the apoptosis rate was more dramatic than others, which indicates that phosphorylation of p53 may play an important role in DPP-induced apoptosis in the shDYRK1B group.

Figure 3. Effect of shDYRK1B on cell cycle arrest in DPP-treated SiHa cells after DYRK1B stably silencing. A. Upper panel: the original pictures; B. Lower panel: the quantification of the population in the G2/M phase in different treated groups by software FlowJo V9; C. Expression of cdc2 and cyclin B1 proteins in DDP-treated SiHa cells. *\( P < 0.05 \) vs normal control group, **\( P < 0.05 \) vs the shScramble group.
shDYRK1B improves DDP sensitivity in cervical cancer

Effect of shDYRK1B on cell invasion and migration in SiHa cells

The influence of DYRK1B knockout on cell migration ability was also detected using a cell wound scratch assay. The results show that after DYRK1B silencing, cell migration ability greatly weakened 60 hours post scratch as shown in Figure 5A. In addition, transwell chambers were used to investigate the change of cell invasion ability. From the experimental results the shDYRK1B group was found to have numbers of cell invasive cells that were greatly decreased compared to the invasive cell number in the shDYRK1B group (42±4). Additionally, this was significantly lower than that of the control group (168±26) and the shScramble group (148±23) (F = 155.9, P < 0.001), as Figure 5B shown. These results were similar with our initial hypothesis, namely that after DYRK1B silencing, the ability of cell invasion obviously would be decreased and the difference would be statistically significant.

Discussion

Cervical cancer is the second most commonly diagnosed cancer and the fourth leading cause of cancer death in women worldwide [12]. Currently, surgical treatment and radiotherapy are dominant methods for cervical cancer patients with chemotherapy as a complementary therapy. It has been reported that the treatment efficiency of conventional chemotherapeutics in cervical cancer is about 50%, and it is even lower for recurrent cervical cancer treatment, complicating with drug resistance and various adverse reaction [13]. The multidrug resistance of chemotherapy drugs remains one of the major clinical obstacles in the successful treatment of cervical cancer. In addition to killing the tumor cells, chemotherapy drugs also do great harm to normal human cells, which largely limits its widely clinical application. It has become one key strategy to increase the sensitivity of human tumor cells to drugs without increasing dosage of the chemotherapy or radiotherapy, and to reduce the cytotoxicity and normal tissue damage induced by drug-treatment.

DDP, a well-known chemotherapeutic drug, is effective against various types of cancers, including carcinomas, germ cell tumors, and sarcomas. Its mode of action has been linked to its ability to crosslink with the purine bases on the DNA, interfering with DNA repair mechanisms, causing DNA damage, and subsequently inducing apoptosis in cancer cells [14]. Meanwhile, DDP also activates some signal transduction pathways involved in cell death, cell repair, and cell cycle within the nucleus and cytoplasm [15]. In our present study, the effects of DYRK1B on SiHa cells sensitivity to DDP in vitro was determined along with the underlying mechanisms through knockdown of DYRK1B by RNAi-mediated gene silencing. The apoptosis rate and sensitivity of cervical cancer SiHa
shDYRK1B improves DDP sensitivity in cervical cancer

The high-risk human papilloma virus (HPV) is detected in 99.7% of cervical cancer patients and is believed to be the causative agent for cervical cancer [16]. DYRK1B is closely associated with high-risk HPV infection and exhibits low-expression in most tissues but overexpression in cervical cancer tissues. Here, in shRNA-infected SiHa cells, protein expression of DYRK1B was significantly down-regulated, indicating that DY RK1B interference could effectively down-regulate expression of the DYRK1B gene in SiHa cells. To evade drug resistance and considerable side effects, combination therapy of DYRK1B inhibitors with other cancer drugs can increase the sensitivity of human tumor cells to chemotherapeutics and does not cause normal cell death in cervical cancer patients.

p53, a crucial tumor suppressor, responds to diverse stress signals by orchestrating specific cellular responses, including transient cell cycle arrest, cellular senescence, and apoptosis, which are all processes associated with tumor suppression [17]. It has been reported that overexpressed p53 can enhance sensitivity of human tumor cells to cisplatin drugs [18]. Similarly, in our present study, we confirmed that suppression of DYRK1B gene expression could increase the sensitivity of SiHa cells to DDP, which is associated with up-regulation of p53 expression and phosphorylation of p53 protein and finally interferes with the p53-dependent apoptosis. Additionally, DYRK1B shRNA interference induced cell cycle arrest in the G2/M phase with a marked up-regulation of cyclin B1 and Cdc2 protein levels. Moreover, recent studies have highlighted roles for p53 in modulating other cellular processes associated with the occurrence and development of cancer, including metabolism, invasion, and metastasis, as well as communication within the tumor microenvironment. Furthermore, the ability of cancer cells to move through the extracellular matrix and invade the surrounding tissue can accelerate the occurrence and development of tumors [19]. Here, our data demonstrate that the ability of cell migration and invasion are all greatly weakened after DYRK1B silencing, which may also be due to upregulation of p53, since p53 functions to suppress metastasis, in part by negatively reg-

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Figure 5. Effect of shDYRK1B on cell invasion and migration in SiHa cells. A. Cell wound scratch assay for cell migration ability determination after DY RK1B knockout. B. Transwell migration assay for cell invasion ability detection after DYRK1B knockout. Scale bar = 50 μm. *P < 0.05 vs normal control group, #P < 0.05 vs the shScramble group. C. The histogram present the number of cells migrated through the membrane of transwell insert by 3 different fields under microscope. Control indicated a blank control group, shScramble indicated a random silenced sequence group, and shDyrk1b was dyrk1b stable silencing group.
shDYRK1B improves DDP sensitivity in cervical cancer

ulating EMT-related programs [20, 21]. These could be another major reasons for the elevated sensitivity of human tumor cells to cisplatin drugs after the stable silencing of DYRK1B. However, to deeply clarify the role of DYRK1B in the development of cervical cancer, the expression levels of some DNA transcription- and repair-related genes needs to be further investigated.

In summary, shRNA can down-regulate expression of the DYRK1B gene, induce human tumor cell growth inhibition and apoptosis by p53 expression regulation, and weaken the ability of cell migration and invasion, increasing the sensitivity of cervical cancer SiHa cells to DDP, and subsequently provide a new approach for cervical cancer chemotherapy.

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Disclosure of conflict of interest

None.

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shDYRK1B improves DDP sensitivity in cervical cancer


