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Cyclin I promotes cisplatin resistance via Cdk5 activation in cervical cancer

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Abstract. – OBJECTIVE: Cisplatin (cis-diamminedichloroplatinum II, CDDP) is one of the most effective chemotherapeutic agents and is widely used in the treatment of cervical cancer (CC), but cancer cell acquired resistance to this drug during the course of its treatment. The aim of this study was to investigate the role of cyclin I to cisplatin resistance in CC cell.

PATIENTS AND METHODS: Cervical tumor specimens from 30 patients were recruited in this study. We analyzed the expression of cyclin I by real-time polymerase chain reaction (qRT-PCR), Western blotting examination of downstream effectors. Cell proliferation assay and xenograft experiments were performed for cisplatin cytotoxicity assay. Lentivirus-mediated and siRNA-mediated genes overexpression or knockdown were applied to investigate the role of cyclin I to cisplatin resistance in CC cell.

RESULTS: We found that high level of cyclin I was associated with cisplatin resistance in CC. Here, we described that cyclin I protein becomes highly expressed in human CC patients resistant to cisplatin chemotherapy. Stable overexpressed cyclin I promotes Hela cell resistance to higher concentrations of cisplatin. In addition, upregulated level of cyclin I increased tumor cells growth *in vitro* and enhanced tumor resistance to cisplatin *in vivo*. The further mechanism investigated showed that cyclin I upregulated the expression of cyclin-dependent kinase 5 (Cdk5) promoting cisplatin resistance by preventing apoptosis in CC cell line. Consistently, the cyclin I overexpressed Hela cell lines produce increased sensitivity to cisplatin treatment through knockdown of Cdk5 protein with siRNA.

CONCLUSIONS: These data suggest that a cyclin I-Cdk5 complex forms a critical antiapoptotic factor in the process of generating cisplatin resistance in cervical cancer.

Key Words:
Cyclin I, Cisplatin resistance, Cdk5, Cervical cancer.

Introduction

Cervical cancer (CC) is the third most common cancer among females worldwide, with the 5-year survival rate is 69%[1]. Surgery represents the mainstay of treatment for patients with early-stage CC, whereas radiotherapy combined with cisplatin-based chemotherapy followed by radical surgery has been reported to significantly improve their overall survival[2,3]. Since patients with locally metastatic or advanced lesions are at significant risk for recurrence, they require concurrent chemoradiation therapy[4]. Cisplatin is the most active agent against CC, with a response rate of 17-21%[5]. Although the overall mortality of patients with CC has decreased over the past few years, CC is a major cause of morbidity and mortality in women. However, emergence of drug-resistant tumor cell remains a major challenge in the treatment of CC. The mechanism of cisplatin resistance is also required further study.

Cyclins were originally discovered for their role in governing cell cycle progression and proliferation[6]. More recently, it has been appreciated that cyclins may influence a wide range of additional cellular functions, including apoptosis, hypertrophy, and differentiation. Cyclin I is the most abundant in post-mitotic tissues. Conversely to the classical cyclins, its level does not fluctuate during the cell cycle[7,8]. More recently found that upregulation of Cyclin I have been involved in cisplatin-resistant[9]. However, the biological function of cyclin I in CC cisplatin-resistant is not known.

In this study, we have investigated the mechanisms that cyclin I play in mediating cisplatin resistance by protecting CC cell from apoptosis via activation of Cdk5. We showed that the protective effects of cyclin I on cisplatin-induced apoptosis failure in cervical cell. Furthermore, we have found that the protective effects of cyclin I on cisplatin was reversed by knockdown the expression of Cdk5.

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Patients and Methods

Cell Lines Culture and Human Tissue Samples

Human cervical cancer cell lines HeLa was procured from American Type Culture Collection (ATCC), The cells were grown in DMEM (Dulbecco's Modified Eagle Medium, Gibco, Grand Island, NY, USA) containing 10% FBS (fetal bovine serum, Sigma-Aldrich, Deisenhofen, Germany) and antibiotics (100 U/ml of penicillin and 100 μg/ml of streptomycin). Cells were maintained as monolayer cultures in a humidified atmosphere of 5% CO_2 at 37°C. For anticancer drug treatment, cells were exposed to 5 μg/ml cisplatin for 12 h. Cisplatin was dissolved in saline before use. After drug exposure, cells were washed and incubated in drug-free medium for 24 h.

Tumor specimens from 30 patients with a histological diagnosis of squamous cervical carcinoma (stage IIIB) and all tumor samples were received the standard radiation therapy with concurrent chemotherapy of weekly cisplatin 40 mg/m^2. Patient included 18 chemoresistant cases and 5 chemosensitive cases according to the pathological and clinical characteristic. Frozen human primary cervical tumor tissues were obtained from surgical resections in accordance with an approved protocol from The Hospital Cancer Center. The study was approved by the Ethics Committee of Henan Province People's Hospital. Written informed consent was obtained from each subject.

Correlates of mRNA Data With Survival

Clinical information including patient age, gender, treatment history, survival, and censoring status was downloaded from The Cancer Genome Atlas (TCGA) portal (https://tcga-data.nci.nih.gov/tcga/tcgaHome2.jsp). Survival differences were investigated between groups of patients with the upper and lower quartile of cyclin I expression. A Kaplan-Meier plot was produced and log-rank testing was performed with survival data.

Transfection of Hela Cells With Cyclin I

To detect the influence of the expression of cisplatin to cisplatin resistance to CC cell line, the cyclin I codig sequence (CDS) was obtained from the National Center for Biotechnology (NCBI). The cyclin I fragment was ligated into mammalian expression vector pLVX-Puro, after sequence verification, the pLVX-cyclin I-Puro or empty vector pLVX-Puro together with pLp1, pLp2 and pLp-VSVG were transfected into 293T packaging cells by using Lipofectamine 2000 reagent (Invitrogen, Shanghai, China) according to the manufacturer's instruction. Lentiviruses in the supernatant were collected and filtered through a 0.45 μm filter. Hela cells were infected with recombinant lentivirus units plus 6 μg/ml polybrene (Sigma-Aldrich, Deisenhofen, Germany). The stable lentivirus infection cells were selected and enriched by puromycin. The entire process was conducted according to the manufacturer's guidelines.

To determine the effect of Cdk5 on CC cell line, siRNA were transfected into cells using Lipofectamine 2000 reagents (Invitrogen, Shanghai, China) according to the manufacturer's instruction.

RNA Extraction and Quantitative Real-Time RT-PCR

The total RNA from patient tissues and Hela cells were extracted using TRIzol reagent (Invitrogen, Shanghai, China) according to the manufacturer's protocol. cDNA was synthesized by using the PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). The primer sequences were listed as follows: cyclin I forward, 5′-AAAGGCCCTGGAAACTTCAT-3′, and reverse, 5′-TGTGAGGCCTACAGCTCTGC -3′; Cdk5 forward, 5′-ATTAGCAGGTTCTGGGGCTT -3′, and reverse, 5′-AATGGTGACCTCGATCCTGA -3′; β-actin forward, 5′- CATTGTAAGAGCCAA-3′, and reverse, 5′-TGAGGTTCGTTGTC-3′; Quantitative RT-PCR (qRT-PCR) analyses were carried out to detect mRNA expression using SYBR Premix Ex Taq™ (TaKaRa, Otsu, Shiga, Japan), and β-actin was used as an internal control. The qRT-PCR reaction was performed on CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) to quantitate the expression levels of Metastasis Suppressor1 (MTSS1) according to the provided protocol, and all qRT-PCR data was normalized to β-actin expression.

Western Blotting

Normal and tumor tissue or Cells were lysed by RIPA (Radio Immunoprecipitation Assay) buffer supplemented with protease inhibitor phenylmethylsulfonyl fluoride (PMSF) on ice, and total proteins (10 mg/lane) were separated on a 10% SDS-PAGE (SDS-polyacrylamide gel

electrophoresis) gel (TaKaRa, Otsu, Shiga, Japan) and transferred to a polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Hercules, CA, USA), after blocked with 5% nonfat milk, the membrane was incubated overnight at 4°C with each primary antibodies against cyclin I (Abcam, Cambridge, UK), Cdk5 (Abcam, Cambridge, UK), activation caspase-3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for apoptosis assay and β-actin (ZhongShan, Beijing, China) as the loading control. Membranes were then incubated with horseradish peroxidase-conjugated secondary antibody at 1:1000 dilution for 2 h at room temperature. Finally, signal detection of the immunoreactive bands was visualized by using ECL reagents (Pierce, Rockford, IL, USA). Signal quantification was performed using the ImageJ program (National Institutes of Health). The specific bands were subjected to densitometry analysis using Quantity One software.

Cisplatin Cytotoxicity Assay

Tumor cells (5000 cells per well) were seeded into 96-well plates and cultured for 6, 12, 18, 24 and 30 h without (Cell proliferation assay) or with various concentrations of cisplatin. At the indicated intervals, 20 mL of Cell Counting Kit-8 (Beyotime, Beijing, China) were added to each well and incubated for 2 hours at 37°C. Thermo Multiskan Spectrum Reader (Thermo Scientific, Schwerte, Germany) was used to measure the absorbance at 540 nm.

Trypan Blue Staining Assay

Cells were stained with trypan blue as described previously[10]. In brief, cells were cultured in 24-well plates; after being collected by trypsinization and suspended in PBS with 0.2% trypan blue (Sigma, St. Louis, MO, USA), the viable cells were counted by hemocytometer.

Tumor Implantation

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Henan Province People's Hospital. For xenograft experiments, each of the 6-week-old male BALB/c nude mice was anesthetized and injected with 1×10^6 Hela cells which stably overexpressing cyclin I or negative control. For cisplatin treatment experiments, when tumors became palpable, cisplatin was intravenously injected into the tail vein of mice bearing either cyclin I expressing tumors or control vector tumors (2 μg per injection; two injections per week). Animals were monitored daily for weight change. After 6 injections, tumor volume was assessed by caliper measurements using the formula (width^2 × length)/2 (mm^3).

Statistical Analysis

All values are expressed as means ± SD. Patients survival was analyzed with Kaplan-Meier method, using the log-rank test for comparison. The statistical significance of differences between two groups was evaluated using Student's *t*-test. Analysis was performed using SPSS version 13.0 software (SPSS, Inc., Chicago, IL, USA). Statistical significance was accepted at $p < 0.05$(*), or $p < 0.01$ (**).

Results

Cyclin I Protein Levels are Elevated in Cisplatin-Resistant Patients and Correlate with Poor Patient Prognosis

Previously studies reported by applied serial analysis of gene expression (SAGE) profiling of cisplatin-resistant and sensitive cells revealed that transcription of cyclin I was upregulated in cisplatin-resistant cells[9]. To further verify this result, we analyzed the protein levels of cyclin I in 4 cisplatin-sensitive and 5 cisplatin-resistant patient tissues obtained from the hospital cancer center. Cyclin I protein levels were significantly elevated in the cisplatin-resistant tumor tissues (Figure 1A, B). To further correlate the levels of cyclin I in cisplatin-resistant cervical cancer specimens, we conducted quantitative real-time PCR analysis of cyclin I expression in 30 patients tumor specimens, including 18 cisplatin tolerance and 12 sensitivities to cisplatin cases. Cyclin I was found to be overexpressed in 18 cases of cisplatin-resistant CC patients (Figure 1C). To determine if different expression of cyclin I could be implicated in patient prognosis, survival data from the The Cancer Genome Atlas (TCGA database were used to evaluate the effects of cyclin I on overall patient survival. The Kaplan-Meier survival curve of patients in the TCGA data with high and low expression of cyclin I showed that overexpession was significantly correlated with reduced patient survival, $p < 0.05$ (Figure 1D). From these data, overexpression of the cyclin I was significantly correlate with reduced survival and cisplatin resistant in CC patients.

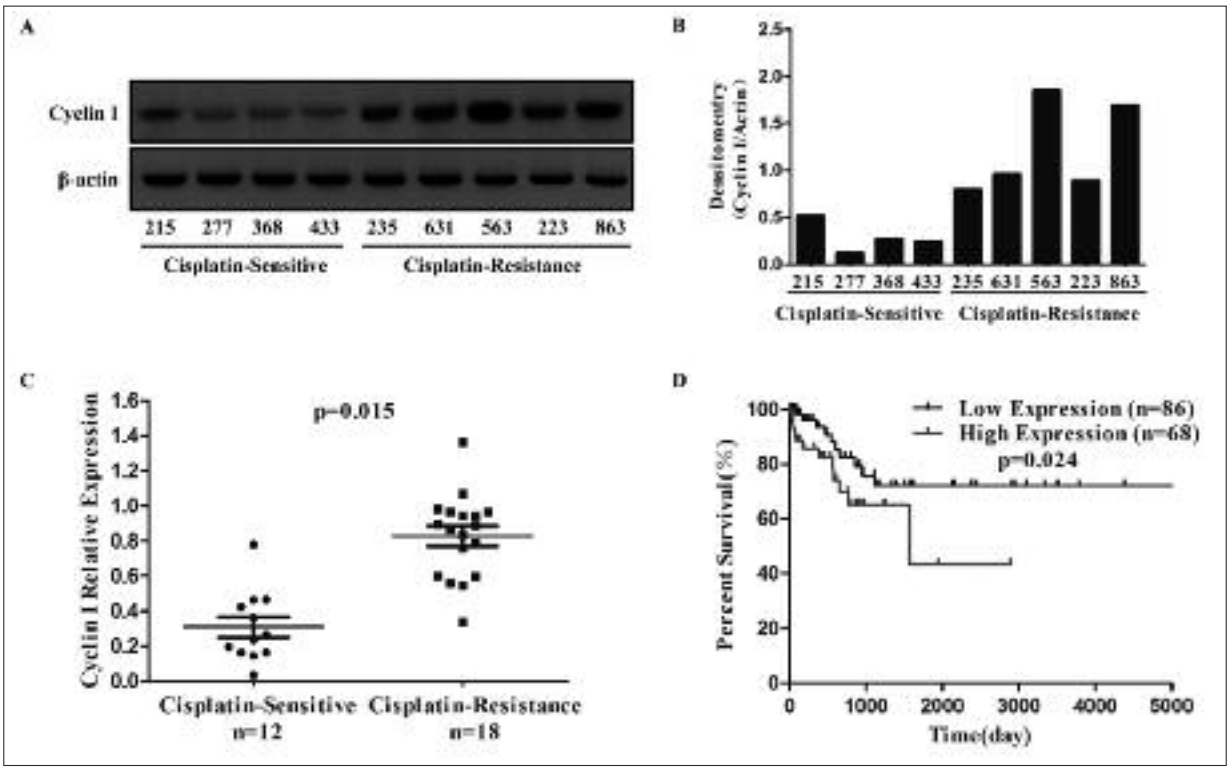

Figure 1. Cyclin I protein is overexpressed in cisplatin-resistant cervical cancer. ***A,-B,*** Cyclin I protein expression in human cervical cancer tissues from cisplatin-sensitive and cisplatin-resistant patients. Patient identifier number is noted in each panel. Nine representative blots were depicted (A). Values presented as the mean of integrated density values cyclin I/Actin (B). ***C,*** qPCR analysis of the mRNA expression levels of cyclin I in 30 cervical cancer patients case (18 cisplatin tolerance and 12 sensitivities to cisplatin cases). High level of cyclin I in cisplatin-resistant patients, $p = 0.015$. ***D,*** Human cervical cancer patient survival is correlated with cyclin I expression. Upregulation of cyclin I correlated with poor patient survival in TCGA database. $p = 0.024$ for decreased patient survival with upregulated cyclin I expression.

Expression of Cyclin I was Induced by Cisplatin and Confer Resistance to Cisplatin

To address the important effect of cyclin I in cervical cisplatin resistant *in vitro*, we utilized the human Hela cell line. We treated Hela cells with different concentration of cisplatin, and induction of cyclin I protein was determined by Western blot analysis. As showed in Figure 2A, cyclin I protein started to increase with increasing concentration of cisplatin, and at a higher level at 6 μg/ml cisplatin. Consistent with induction of cyclin I protein, cyclin I mRNA was increased on cisplatin treatment in Hela cells (Figure 2B), which suggests that cisplatin induces cyclin I through a transcriptional mechanism. To investigate whether cyclin I is involved in cisplatin resistance *in vitro*, cells were infected with Lentivirus carrying pLVX which overexpressing cyclin I (pLVX-cyclin I) or empty vector control (pLVX) transfected with empty vector and were subsequently selected. The cell death was examined when the cells were incubated in medium containing 2 μg/ml and 5 μg/ml cisplatin for 12 hours later. Hela cells expressing cyclin I (pLVX-cyclin I) was significantly more resistant to cisplatin than control Hela cells (pLVX) and Hela cells (Figure 2C). Then, the cell survivals were determined 30 hours later with exposed to 5 μg/ml cisplatin. As expected, cells expressing cyclin I (pLVX-cyclin I) was significantly more resistant to cisplatin treatment than cell no transfected or control cells (pLVX) (Figure 2D). Thus, our results indicate that expression of cyclin I confers resistance to cisplatin in CC cell.

Cyclin I Promote Cervical Cancer Resistance to Cisplatin in vivo

To test the role of cyclin I in CC growth and cisplatin resistance *in vivo*, we used hela cell models in nude mice. First, to determine the impact of cyclin I on CC cell proliferation *in vivo*,

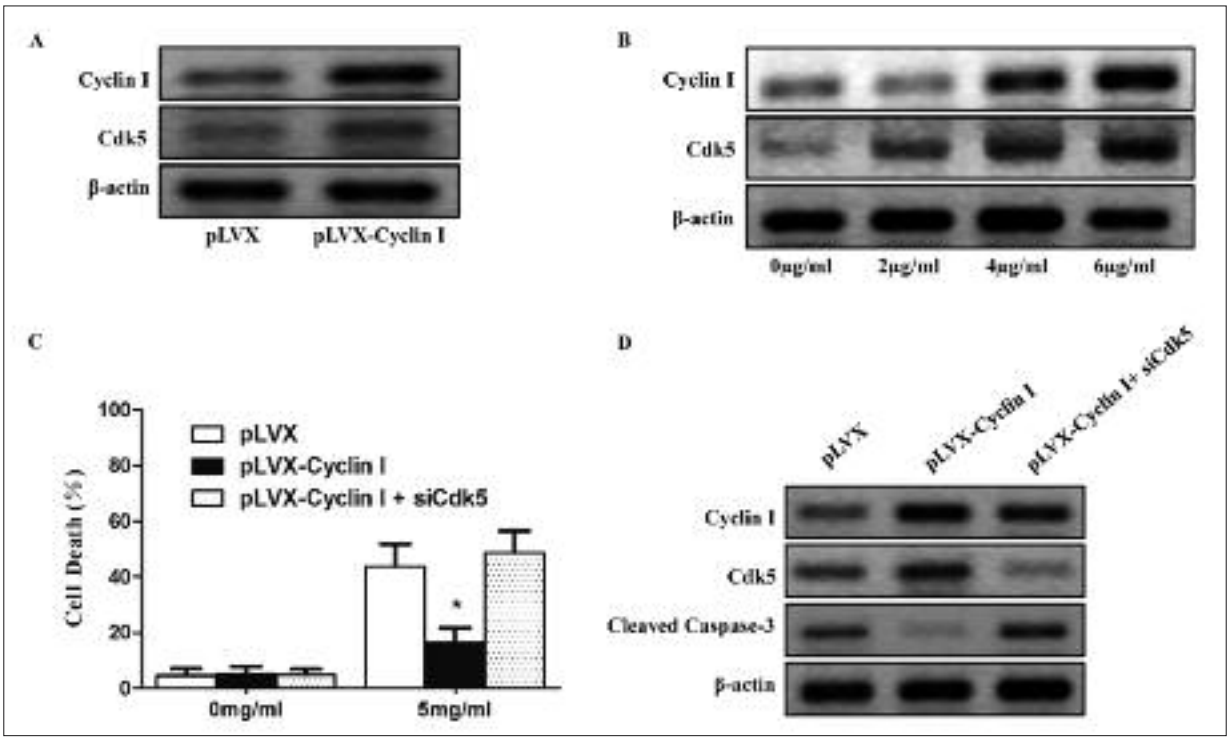

Figure 2. Cyclin I expression confers resistance to cisplatin in cervical cancer cell lines. ***A,*** The expression of cyclin I in Hela cell treated with different concentrations cisplatin after 12 hours later, β-actin was used as loading control. ***B,*** The mRNA levels of cyclin I in hela cell after 12h treatment with cisplatin at the indicated concentrations. ***C,*** Dead cell counts were determined in Hela cells were treated with 5 μg/ml after 12 hours by using the trypan blue exclusion assay. Columns, mean; bars, SD; $*p < 0.05$. ***D,*** Cells exposure to different concentration cisplatin and cell survival was measured by CCK8 assay. All experiments were repeated at least thrice.

we showed overexpressing cyclin I promote tumor cells growth compared to pLVX or Hela cells (Figure 3A). We then inoculated mice with cervical cell to test the cisplatin therapeutic effect in the xenograft model. Mice inoculated with the Hela cell with overexpession of cyclin I or empty vector were all treated with cisplatin. All mice inoculated with overexpression of cyclin I cell had significantly larger tumors than mice inoculated control cell (Figure 3B and C). Overexpression of cyclin I also led to a significant increase in tumor weight (Figure 3D). Thus, overexpression of cyclin I enhanced the chemoresistance of CC *in vivo*.

Cyclin I Activate Cdk5 and Protect Cervical Cancer cells from Apoptosis

Recently reported that a cyclin I-Cdk5 complex forms a critical antiapoptotic factor in terminally differentiated cells[11]. To explore the biological mechanisms for the role of cyclin I in cisplatin resistant cells, we investigated where cyclin I confer cisplatin resistance by activated Cdk5 protects CC cells from apoptosis. First, to determine if cyclin I have also activated Cdk5 in CC cell lines, as shown in Figure 4A, overexpressing cyclin I upregulated the protein level of Cdk5. Furthermore, we compared cyclin I effect to expression of Cdk5 in Hela cells treated with different concentration cisplatin. We found that the expression of Cdk5 was increased with the expression of cyclin I via concentration dependence (Figure 4B). To test the hypothesis that activation of Cdk5 by cyclin I was necessary for cisplatin resistance, we were inhibiting Cdk5 activity with siRNA in overexpressing cyclin I Hela cell and detection by cleaved caspase-3 in cisplatin explored. Cell death assay indicated that lowering Cdk5 was more sensitive to cisplatin in CC cells (Figure 4C). Further mechanism investigation suggested that cisplatin-induced apoptosis was inhibited by cyclin I-Cdk5 in Hela cells, as indicated caspase-3 activity observed in Figure 4D. These data show that active Cdk5 by cyclin I was necessary to cisplatin resistance via reduce apoptosis.

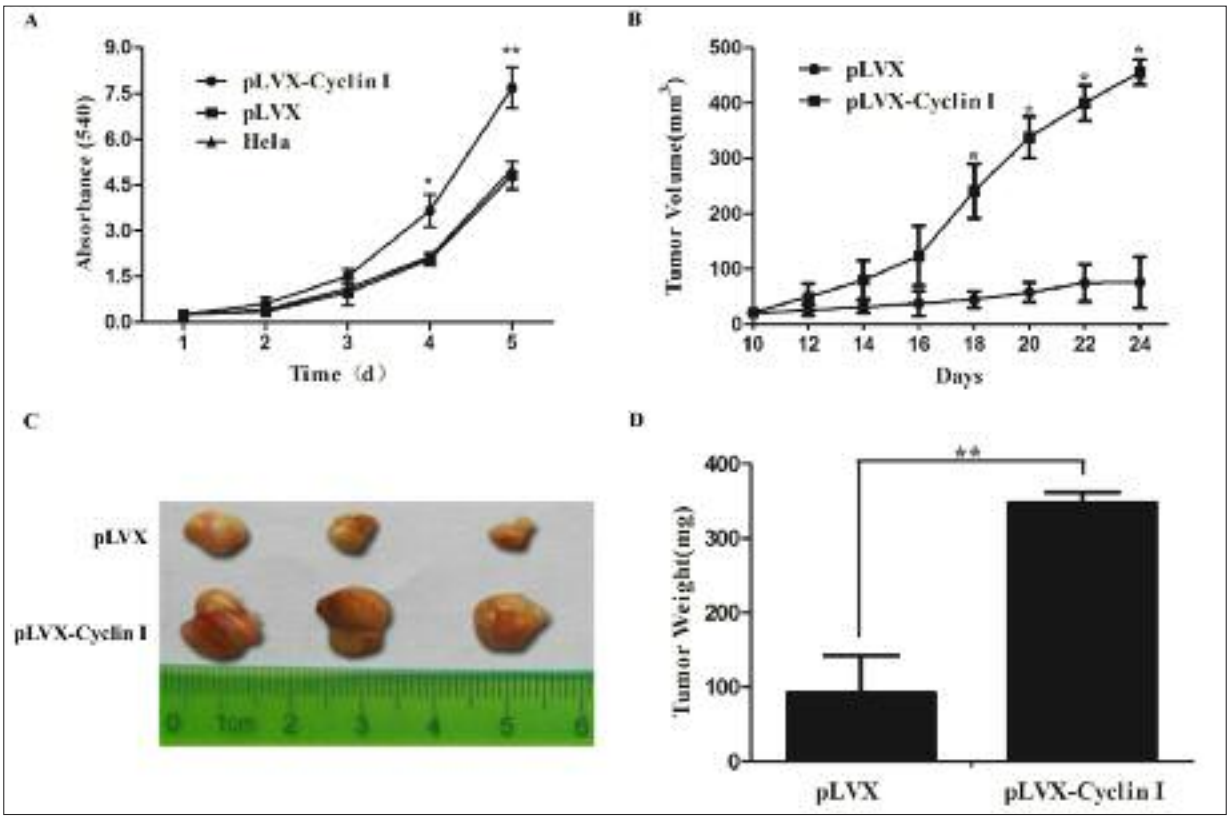

Figure 3. The *in vivo* effects of cyclin I on the cisplatin treated cervical cancer cells. ***A,*** Overexpression of cyclin I increased growth rate in Hela cell lines *in vitro*. The growth curve comparing the rate of proliferation of Hela cells with cyclin I overexpression and control vector or Hela cells. ***B,*** Tumor volume at different time points after subcutaneous transplantation and treated with cisplatin. Each point represents the mean ± SD of results obtained from 3 mice. ***C,*** Photographs of tumors formed at 24 days after the subcutaneous transplantation were shown. ***D,*** Tumor weights between the pLVX and the pLVX-Cyclin I group24 day after the subcutaneous transplantation.

Discussion

Cisplatin binds to and causes cross-linking of DNA and induce DNA damage, which leads either to cell cycle arrest or immediate activation of apoptosis and killing of cancer cells[12]. Cisplatin has been the standard cytotoxic agent for the treatment of advanced cervical cancer[13]. However, the major limitation of cisplatin in clinical cancer treatment is due to resistance. Studied to the mechanism of cisplatin resistance shown that cancer cells can develop cisplatin resistance through a variety of mechanisms, including reduced intracellular accumulation of chemotherapy drug, increased levels of glutathione, altered activation of signaling pathways and changes in the apoptotic cell death pathways[14,15]. Earlier studies have indicated that cisplatin-resistant in CC were associated with the nucleotide excision repair gene, increase of NFκB activation by MEK-ERK signaling pathway and the up-regulated anti-apoptotic protein[16-18]. In the current study, we show that high level of Cyclin I confers CC cell resistance to cisplatin by activates Cdk5.

The results of the present study show that cyclin I protein was high expressing in the CC patients with resistant to cisplatin treatment, and high levels of cyclin I was associated with poor clinical survival (Figure 1). Our findings, therefore, consistent with several earlier studies include that elevated expression of cyclin I in ovarian cancer and in invasive human breast cancers[19,20], indicating that elevated cyclin I levels are associated with the occurrence of the tumor. Also we founded that the expression of cyclin I can be induced by cisplatin (Figure 2). Similarly, cyclin I was highly upregulated gene in cisplatin-induced ovarian cancer cells[9]. These collective results suggest that we hypothesized the expression of cyclin I may play a key role in cisplatin-

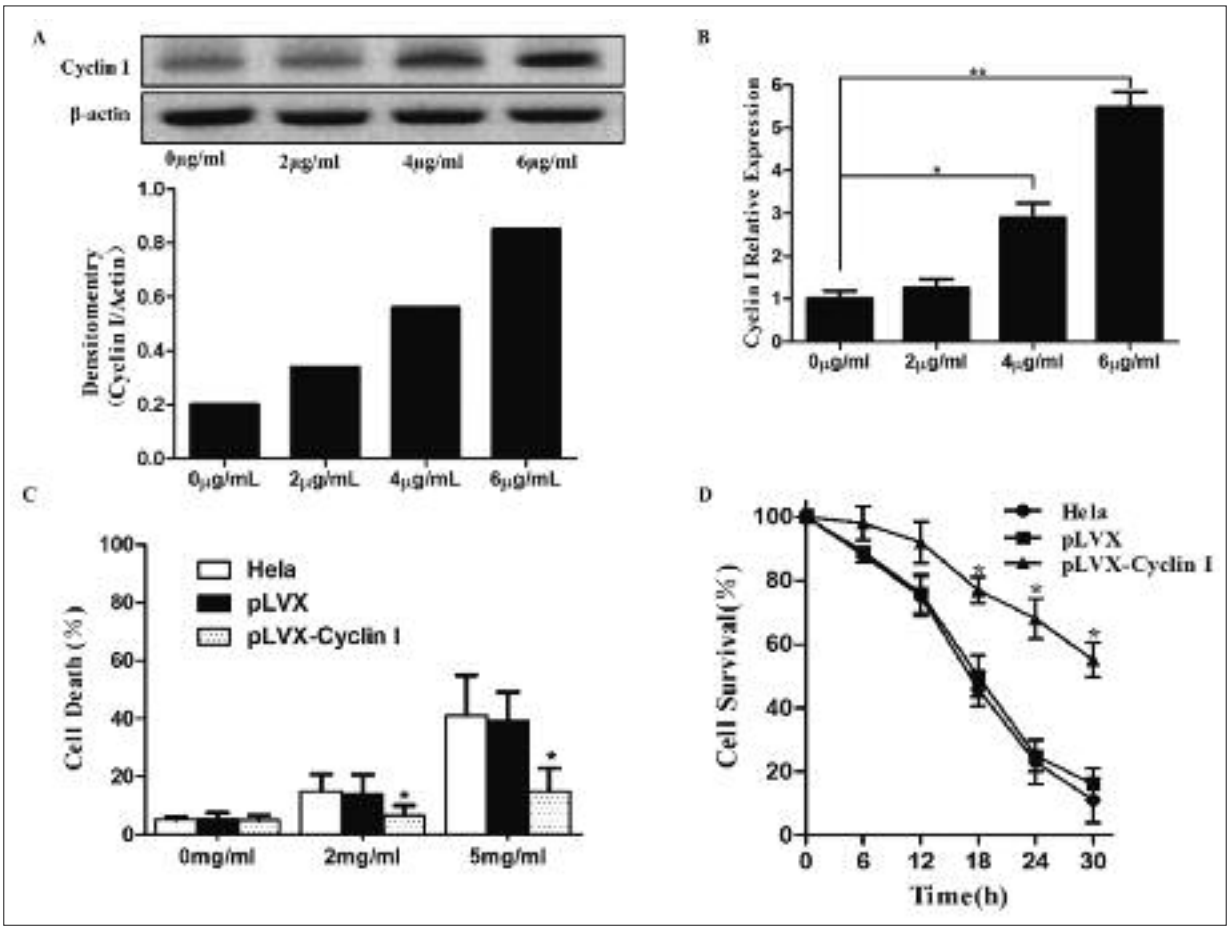

Figure 4. Cdk5 activity is required to cisplatin-resistant by decreased apoptosis. ***A,*** Western blots detection of the expression of Cdk5. The increase expression of Cdk5 in overexpressing cyclin I (pLVX-cyclin I) compared with control (pLVX) in Hela cells. ***B,*** The expression of cyclin I and Cdk5 can be induced by different concentration cisplatin. ***C,*** siRNAs silenced Cdk5 expression and restored the chemosensitive of Hela cells transfected with the cyclin I. Significant differences were observed in silenced Cdk5 by trypan blue exclusion assay ($p < 0.05$). ***D,*** Western blot analysis indicated that silenced Cdk5 enhanced the cisplatin-induced apoptosis as showed the upregulated expression of caspase-3 activity in pLVX-Cyclin I Hela cells.

resistant patients. Consistent with the expected, we found that overexpression of cyclin I confers resistance to cisplatin in CC cell lines (Figure 2). It is believed that cyclin I has no role in regulating cell proliferation because cyclin I is expressed at high levels in post-mitotic tissues, and its level does not fluctuate during the cell cycle[8,21,22]. However, in the current study, cell proliferation and tumor growth indicated that upregulated levels of cyclin I promote cell proliferation *in vitro* and promote the tour grow in treated with cisplatin *in vivo* (Figure 3). This observation agrees that higher cyclin I was significantly correlated with ovarian cancer cells proliferative activity[19]. There was a trend suggesting cyclin I plays a critical role in cisplatin resistant in CC.

The mechanistic basis for the association between high cyclin I level and cisplatin resistance will require further investigation. It was reported that cyclin I may be post -transcriptionally regulated by ubiquitination[23] and dysregulation of ubiquitin-mediated degradation of cyclin I may be related to aberrant cell cycle regulation and/or resistance to apoptosis, which are characteristic of invasive breast and other tumors[20]. In this study, we discovered activity Cdk5 regulated by cyclim I was required in the cisplatin resistance CC (Figure 4).

Cdk5 is a unique member of a small serine/threonine cyclin-dependent kinase family[24]. Cdk5 has versatile biological functions in cells discovered in recent decades. CDK5 is acti-

vated by the association with obligate CDK5-specific activator proteins, p35, p39[25], and p67[26]. Cdk5/p35 and p25 are novel players in digoxin-triggered prostate cancer cell apoptosis[27]. p35 as an activator of Cdk5 protects podocytes against apoptosis *in vitro* and *in vivo*[22]. Moreover, cyclin I as a novel regulator of Cdk5 reduce apoptosis was reported in neurons[28]. Cyclin I also be reported protects podocytes from apoptosis[21]. In our study, Cdk5 was activated by cyclin I and increased Cdk5 expressing was observed in CC cell line with cisplatin treatment. To investigate the role of Cdk5 in cisplatin-resistant, Cdk5 was inhibited by using siRNA. The results indicated that Cdk5 was necessary in cisplatin resistance caused by cyclin I in CC cell line (Figure 4). Further investigation was focused on how Cdk5 result of cisplatin-resistant.

Conclusions

We provided evidence that cyclin I-Cdk5 confer CC cell resistance to cisplatin. We have also demonstrated the expression of cyclin I level associated with patient survival. It is noteworthy that Cdk5 activity by cyclin I was necessary to confer cervical cell resistance to cisplatin. These results indicated that the cyclin I may become a novel target for anticancer therapy.

Acknowledgements

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013; 63: 11-30.
2) Mancuso S, Smaniotto D, Benedetti Panici P, Favale B, Greggi S, Manfredi R, Margariti PA, Morganti AG, Scambia G, Tortoreto F, Valentini V, Cellini N. Phase I-II trial of preoperative chemoradiation in locally advanced cervical carcinoma. Gynecol Oncol 2000; 78(3 Pt 1): 324-328.
3) Thomas GM. Improved treatment for cervical cancer--concurrent chemotherapy and radiotherapy. N Engl J Med 1999; 340: 1198-1200.
4) Im SS, Monk BJ. New developments in the treatment of invasive cervical cancer. Obstet Gynecol Clin North Am 2002; 29: 659-672.
5) Bonomi P, Blessing JA, Stehman FB, DiSaia PJ, Walton L, Major FJ. Randomized trial of three cisplatin dose schedules in squamous-cell carcinoma of the cervix: a gynecologic Oncology Group study. J Clin Oncol 1985; 3: 1079-1085.
6) Evans T, Rosenthal ET, Youngblom J, Distel D, Hunt T. Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. Cell 1983; 33: 389-396.
7) Nakamura T, Sanokawa R, Sasaki YF, Ayusawa D, Oishi M, Mori N. Cyclin I: a new cyclin encoded by a gene isolated from human brain. Exp Cell Res 1995; 221: 534-542.
8) Jensen MR, Audolfsson T, Factor VM, Thorgeirsson SS. In vivo expression and genomic organization of the mouse cyclin I gene (Ccni). Gene 2000; 256: 59-67.
9) Sherman-Baust CA, Weeraratna AT, Rangel LB, Pizer ES, Cho KR, Schwartz DR, Shock T, Morin PJ. Remodeling of the extracellular matrix through overexpression of collagen VI contributes to cisplatin resistance in ovarian cancer cells. Cancer Cell 2003; 3: 377-386.
10) Malmberg J, Tolmachev V, Orlova A. Imaging agents for in vivo molecular profiling of disseminated prostate cancer: cellular processing of [(111)In]-labeled CHX-A"DTPA-trastuzumab and anti-HER2 ABY-025 Affibody in prostate cancer cell lines. Exp Ther Med 2011; 2: 523-528.
11) Brinkkoetter PT, Olivier P, Wu JS, Henderson S, Krofft RD, Pippin JW, Hockenbery D, Roberts JM, Shankland SJ. Cyclin I activates Cdk5 and regulates expression of Bcl-2 and Bcl-XL in postmitotic mouse cells. J Clin Invest 2009; 119: 3089-3101.
12) Mandic A, Hansson J, Linder S, Shoshan MC. Cisplatin induces endoplasmic reticulum stress and nucleus-independent apoptotic signaling. J Biol Chem 2003; 278: 9100-9106.
13) Robati M, Holtz D, Dunton CJ. A review of topotecan in combination chemotherapy for advanced cervical cancer. Ther Clin Risk Manag 2008; 4: 213-218.
14) Koberle B, Tomicic MT, Usanova S, Kaina B. Cisplatin resistance: preclinical findings and clinical implications. Biochim Biophys Acta 2010; 1806: 172-182.
15) Selvendiran K, Ahmed S, Dayton A, Kuppusamy ML, Rivera BK, Kalai T, Hideg K, Kuppusamy P. HO-3867, a curcumin analog, sensitizes cisplatin-resistant ovarian carcinoma, leading to therapeutic synergy through STAT3 inhibition. Cancer Biol Ther 2011; 12: 837-845.
16) Castagna A, Antonioli P, Astner H, Hamdan M, Righetti SC, Perego P, Zunino F, Righetti PG. A proteomic approach to cisplatin resistance in the cervix squamous cell carcinoma cell line A431. Proteomics 2004; 4: 3246-3267.

17) Yeh PY, Chuang SE, Yeh KH, Song YC, Ea CK, Cheng AL. Increase of the resistance of human cervical carcinoma cells to cisplatin by inhibition of the MEK to ERK signaling pathway partly via enhancement of anticancer drug-induced NF kappa B activation. Biochem Pharmacol 2002; 63: 1423-1430.

18) Britten RA, Liu D, Tessier A, Hutchison MJ, Murray D. ERCC1 expression as a molecular marker of cisplatin resistance in human cervical tumor cells. Int J Cancer 2000; 89: 453-457.

19) Cybulski M, Jarosz B, Nowakowski A, Jeleniewicz W, Seroczynski P, Mazurek-Kociubowska M. Cyclin I correlates with VEGFR-2 and cell proliferation in human epithelial ovarian cancer. Gynecol Oncol 2012; 127: 217-222.

20) Landberg G, Nilsson K, Jirstrom K, Ryden L, Kitching R, Burger AM, Seth A. Cyclin I is expressed in human breast cancer and closely associated with VEGF and KDR expression. Breast Cancer Res Treat 2005; 89: 313-316.

21) Griffin SV, Olivier JP, Pippin JW, Roberts JM, Shankland SJ. Cyclin I protects podocytes from apoptosis. J Biol Chem 2006; 281: 28048-28057.

22) Brinkkoetter PT, Wu JS, Ohse T, Krofft RD, Schermer B, Benzing T, Pippin JW, Shankland SJ. p35, the non-cyclin activator of Cdk5, protects podocytes against apoptosis *in vitro* and *in vivo*. Kidney Int 2010; 77: 690-699.

23) Seth A, Kitching R, Landberg G, Xu J, Zubovits J, Burger AM. Gene expression profiling of ductal carcinomas in situ and invasive breast tumors. Anticancer Res 2003; 23: 2043-2051.

24) Demetrick DJ, Zhang H, Beach DH. Chromosomal mapping of human CDK2, CDK4, and CDK5 cell cycle kinase genes. Cytogenet Cell Genet 1994; 66:72-74.

25) Dhavan R and Tsai LH. A decade of CDK5. Nature reviews Mol Cell Biol 2001; 2: 749-759.

26) Shetty KT, Kaech S, Link WT, Jaffe H, Flores CM, Wray S, Pant HC, Beushausen S. Molecular characterization of a neuronal-specific protein that stimulates the activity of Cdk5. J Neurochem 1995; 64: 1988-1995.

27) Lin H, Juang JL, Wang PS. Involvement of Cdk5/p25 in digoxin-triggered prostate cancer cell apoptosis. J Biol Chem 2004; 279: 29302-29307.

28) Weishaupt JH, Neusch C, Bahr M. Cyclin-dependent kinase 5 (CDK5) and neuronal cell death. Cell Tissue Res 2003; 312: 1-8.