Int J Clin Exp Pathol 2014;7(7):4016-4023
www.ijcep.com /ISSN:1936-2625/IJCEP0000792

Original Article

Cyclin D1b overexpression inhibits cell proliferation and induces cell apoptosis in cervical cancer cells *in vitro* and *in vivo*

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Received May 14, 2014; Accepted June 27, 2014; Epub June 15, 2014; Published July 1, 2014

Abstract: Cyclin D1b is one of two proteins translated from cyclin D1 transcripts (isoforms a and b) that are generated due to gene polymorphism. Our previous study has reported low cyclin D1b expression in cervical cancer tissue, with an expression level in moderately or poorly differentiated tissues that was significantly lower than that in well-differentiated tissues. However, the functional role of cyclin D1b in cervical cancer remains to be elucidated. In this study, using a cervical cancer cell line with stable expression of cyclin D1b, we found that upregulation of cyclin D1b initiated cell cycle arrest at the G0/G1 phase and induced apoptosis, thereby inhibiting cell proliferation and colony formation. Furthermore, xenograft transplantation experiments in nude mice demonstrated that cyclin D1b upregulation inhibited cancer growth and induce apoptosis in vivo. In conclusion, the present study indicates anti-tumor effects of cyclin D1b in cervical cancer, suggesting that cyclin D1b may represent a potential therapeutic target for cervical cancer.

Keywords: Cyclin D1b, cervical cancer, proliferation, apoptosis

Introduction

Cervical cancer is a common carcinoma of the female reproductive system that has the second highest incidence and the fifth highest mortality among all carcinomas [1]. More than 80% of cervical cancers worldwide occur in developing countries [2], and approximately 150,000 new cases are diagnosed in China each year, accounting for 30% of all new cases globally [3]. It is generally accepted that human papillomavirus (HPV) infection is the most important factor responsible for cervical cancer. However, only a small number of HPV-infected patients develop cervical cancer [4], indicating that the occurrence of cervical cancer is a complex process that involves numerous factors. To date, therapeutic strategies for cervical cancer mainly include surgical treatment, radiotherapy and chemotherapy. However, all of these treatments have limitations [5], and improving the survival rate of patients remains a challenging task. Thus, further studies on mechanisms of cervical cancer and the identification of new and effective gene therapy targets are of great significance to the development of new treatment strategies and the improvement of patient survival.

Cyclin D1 is a key regulator of the cell cycle G1-S transition, and previous studies have shown that aberrant expression of cyclin D1 is closely associated with a variety of tumors [6-8]. The CCND1 gene encoding cyclin D1 contains a single nucleotide polymorphism locus (DNA rs9344), and polymorphisms at this location can lead to alternative splicing and the generation of two different transcripts that are translated into two proteins, cyclin D1a and cyclin D1b [9]. Currently, the mechanisms underlying the function of cyclin D1a, which is the classic type of cyclin D1, are thoroughly understood, whereas few studies on cyclin D1b have been reported due to its recent discovery. To date, the role of cyclin D1b in malignant tumors remains highly controversial. For example, studies in prostate cancer [10] and bladder cancer [11] indicated that cyclin D1b is an oncogene,

while studies in breast cancer cells showed that cyclin D1b antagonizes the role of cyclin D1a and inhibits tumor growth [12]. In addition, other studies suggest that cyclin D1b does not affect the pathogenesis of multiple myeloma and mantle cell lymphoma [13]. Our previous clinical study found that the expression of cyclin D1b was downregulated in cervical cancer [14]. Therefore, we speculated that the role of cyclin D1b may vary in different tumors, although the underlying mechanisms remain unclear.

In the present study, we established a cervical cancer cell line that stably overexpressed cyclin D1b and investigated the effects of cyclin D1b on the proliferation and apoptosis of cervical cancer cells in vitro and in vivo. The results showed that cyclin D1b overexpression inhibited cervical cancer cell proliferation and induced apoptosis. Therefore, cyclin D1b may play an important regulatory role in the occurrence and development of cervical cancer.

Materials and methods

Cell culture

The human cervical carcinoma HeLa cells were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS, HyClone, Logan, UT, USA), 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma-Aldrich, St. Louis, MO, USA) and grown at 37°C in an incubator with 5% CO_2. The medium was replaced once every 2-3 days, and cells were digested with 0.25% trypsin and passaged when they reached 80% confluence.

Plasmid construction and stable transfection

The full-length coding sequence of human cyclin D1b gene was amplified by reverse transcription polymerase chain reaction (RT-PCR) and ligated into the pEGFP-C1 plasmid (Clontech, Cambridge, UK) at the EcoR/XhoI restriction enzyme recognition sites. The constructed plasmid was then sequenced and designated as pEGFP-cyclin D1b. HeLa cells were seeded in 6-well plates at a density of 1.0×10^5 cells/well and cultured overnight. When the cells grew to 70-80% confluence, EGFP-cyclin D1b and pEGFP-C1 empty vector were transfected using Lipofectamine 2000™ (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Forty-eight hours after transfection, geneticin (G418, Invitrogen) at a concentration of 800 μg/ml was added to screen stably transfected cell lines. Four weeks later, monoclonal cells with G418 resistance were selected, and the expression level of cyclin D1b was determined. Next, the selected cells were continuously cultured and passaged in medium containing G418 (350 μg/ml) for subsequent experiments.

Real-time PCR

Total RNA was extracted from cells using TRIzol reagent (Invitrogen) and reverse transcribed to cDNA using a first-strand cDNA synthesis kit (Takara, Dalian, China) according to the manufacturer's instructions. The primer sequences are as follows: cyclin D1b, forward: 5'-AACAGATCATCCGCAAACAC-3', reverse: 5'-TGGGACATCACCCTCACTTA-3'; β-actin, forward: 5'-GGAGATTACTGCCCTGGCTCCTAGC-3', reverse: 5'-GGCCGGACTCATCGTACTCCTGCTT-3'. Real-time PCR was conducted on the Exicycler™ 96 fluorescence quantitative instrument (Bioneer, Daejeon, Korea). The total volume of the reaction system was 20 μl, consisting of 1 μl of cDNA, 0.5 μl of each of the upstream and downstream primers, 10 μl of SYBR Green master mix and 8 μl of ddH_2O. The PCR reaction program was as follows: 95°C for 10 min; 40 cycles of 95°C for 10 s, 58°C for 20 s, and 72°C for 30 s; and then 4°C for 5 min.

Western blot analysis

Cells were lysed using RIPA lysis buffer (Beyotime Institute of Biotechnology, Haimen, China), and the protein concentrations were quantified using the bicinchoninic acid (BCA) assay method. Equal amounts of proteins (40 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA). The membrane was blocked with 5% nonfat milk at room temperature for 1 h and then incubated with anti-cyclin D1b antibody (1:500, Wanlei Life Science, Shenyang, China) at 4°C overnight, followed by incubation with horseradish peroxidase (HRP)-labeled secondary antibody (1:5,000, Beyotime Institute of Biotechnology) at 37°C for 1 h. The blotted protein bands were exposed to and visualized using enhanced chemiluminescence (ECL)

reagent. Developed films were digitized by scanning, and the optical densities were analyzed with the Image J software. β-actin was used as an internal reference for the analysis of protein expression.

Cell proliferation assay

Cell proliferation was assessed using the 3-(4,5-dimethyl-2)-2,5-diphenyl tetrazolium bromide (MTT) assay. Cells were seeded into 96-well plates at a density of 5×10^3/well and placed in an incubator until the cells grew to confluence. Subsequently, 100 μl of MTT solution (0.5 mg/ml, Sigma-Aldrich) was added at different time points, and then the samples were incubated at 37°C for 4 h. After the supernatant was aspirated, 200 μl of dimethyl sulfoxide (DMSO, Sigma-Aldrich) was added to dissolve formazan crystals formed in the cells. The absorbance value of each well was measured at 490 nm using a microplate reader.

Colony formation assay

Cells were seeded into 10-mm dishes at a density of 2×10^2 cells/dish and then cultured at 37°C for 10-14 days until the visible cell clones were formed. Afterward, the cells were fixed with methanol. Fixative buffer was discarded, and the cells were stained with Giemsa dye for 10 min. After the staining solution was discarded, the number of cell clones was counted.

Detection of cell cycle and apoptosis with flow cytometry

For cell cycle analysis, cells were digested with trypsin and fixed overnight with ice-cold 70% ethanol. Next, 50 μg/ml propidium iodide (PI) containing RNase enzymes was added, and the cells were incubated at 37°C for 30 min. The proportions of cells in each cycling stage were detected using flow cytometry (BD Biosciences, San Jose, CA, USA).

An annexin V-FITC/PI apoptosis detection kit (KeyGen Biotech, Nanjing, China) was used to detect cell apoptosis. At 80% confluence, the cells were trypsinized and collected. After washing with PBS, 500 μl of annexin V-binding buffer was added to resuspend the cells. After the addition of 5 μl FITC-labeled annexin V and 5 μl PI, the cells were incubated at room temperature in the dark for 15 min and then subjected to detection by flow cytometry.

Xenograft transplantation into nude mice

Six-week-old female nude mice (BALB/c nude) were purchased from the Experimental Animal Center of China Medical University. The care and use of the experimental animals was approved by the Institutional Animal Ethics Committee of China Medical University prior to the study. A total of 1×10^7 cells in the logarithmic growth phase were collected and injected into nude mice at the right forelimb armpit, with 8 mice in each group. The long- and short-axis diameters of the tumors were measured every 3 days starting on day 12 post-injection, and the tumor volume (V) was calculated using the formula $V = ab^2/2$ (a and b represent the long and short-axis diameters of the tumor, respectively). At 30 days after inoculation, the mice were sacrificed, and the tumors were removed for subsequent experiments.

Terminal transferase-mediated biotin dUTP nick end labeling (TUNEL) assay

Cell apoptosis was evaluated using the Colorimetric TUNEL Apoptosis Assay Kit (Beyotime Institute of Biotechnology) following the manufacturer's instructions. After inactivation using H_2O_2 solution, cell-seeded slides or paraffin-embedded sections were incubated with 50 μl of biotin-labeled solution at 37°C for 60 min. Following wash with PBS, 50 μl of streptavidin-HRP working solution was added, and the slide/sections were incubated at room temperature for 30 min. Following 3, 3-diaminobenzidine (DAB) development, hematoxylin was used to stain the nuclei, and the slide/sections were photographed under a microscope.

Statistical analysis

Data are presented as the mean ± standard deviation and were analyzed using one-way analysis of variance between groups using the SPSS 16.0 software. The Bonferroni post-hoc test was used for multiple comparison, and $P < 0.05$ was considered statistically significant.

Results

Establishment of HeLa cells stably overexpressing cyclin D1b

To investigate the role of cyclin D1b in cervical cancer, we constructed the cyclin D1b expression plasmid pEGFP-cyclin D1b and then trans-

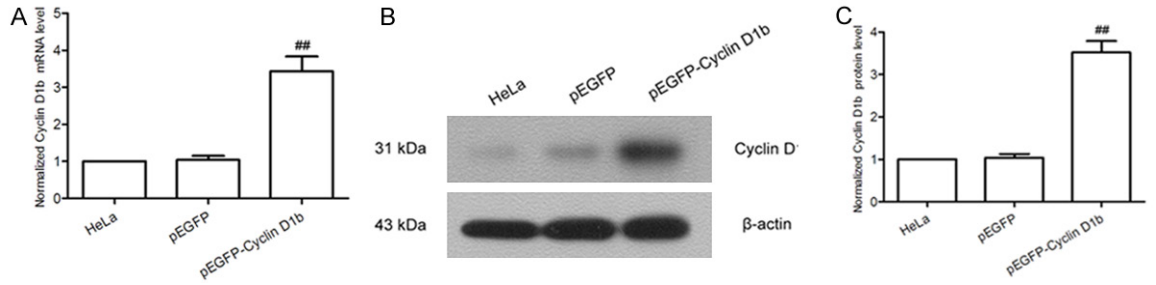

Figure 1. Expression of cyclin D1b in stably transfected HeLa cells. A. Real-time PCR was used to detect the cyclin D1b mRNA expression level. B. Western blotting was used to detect the cyclin D1b protein expression level in each group. Representative results obtained from three repeated experiments are presented in the figure. C. β-actin was used as an internal reference to analyze the relative protein expression level of cyclin D1b, n = 3. ##P < 0.01, compared with the control group.

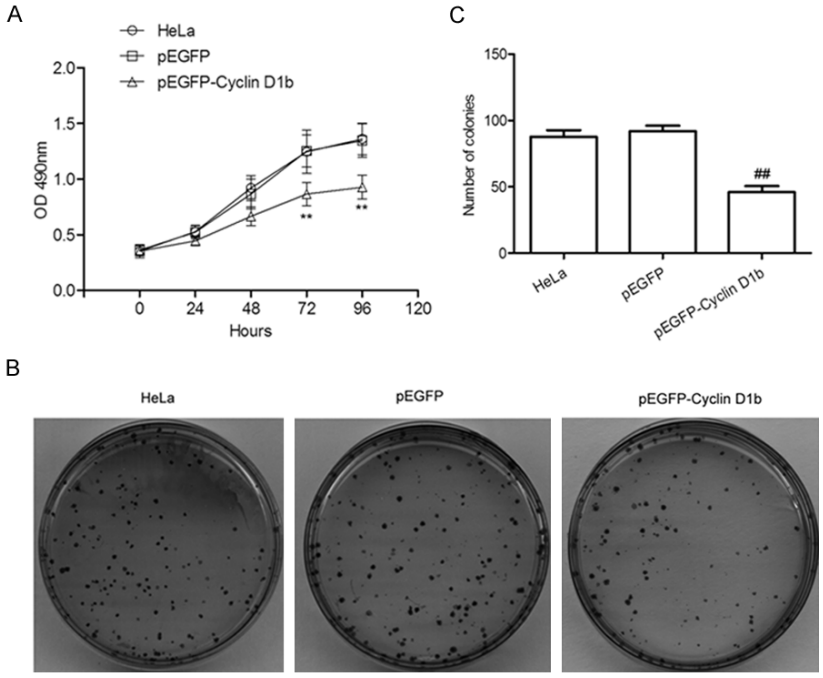

Figure 2. Upregulation of cyclin D1b inhibited the proliferation and colony formation of HeLa cells. A. The MTT method was used to detect cell proliferation. Cells were seeded in 96-well plates, and the absorbance of each well was detected at different time points at 490 nm, n = 6. B. Colony formation was assayed to evaluated cell colony-forming ability. The cells were seeded in 10-mm Petri dishes at a density of 2×10^2 cells/dish, and after 10-14 days, colony formation was observed. Representative results from each group are shown in the figure. C. Comparison of colony formation between the groups, n = 3. $^{**}P < 0.01$, compared with the control group; $^{\#\#}P < 0.01$, compared with the pEGFP-C1 group.

fected pEGFP-cyclin D1b and pEGFP-C1 into HeLa cells. G418-resistant cell clones were then selected, and real-time PCR and Western blot analysis were used to detect the expression of cyclin D1b. Significantly increased mRNA and protein expression levels of cyclin D1b were observed in the pEGFP-cyclin D1b-transfected group compared to the control group (**Figure 1**; $P < 0.01$), indicating that HeLa cervical cancer cells stably overexpressing cyclin D1b were established successfully.

Upregulation of cyclin D 1b inhibited the proliferation and colony formation of cervical cancer cells

The MTT assay was used to detect the effect of cyclin D1b upregulation on HeLa cell proliferation. As shown in **Figure 2A**, the proliferation of pEGFP-cyclin D1b-transfected cells was significantly suppressed 48 h after seeding and was also significantly decreased at 72 h and 96 h after seeding compared to the control group ($P < 0.01$). The colony-forming ability of the cells was also measured to evaluate the effect of cyclin D1b on HeLa cell tumorigenicity in vitro. The result showed that the number of colonies formed was significantly reduced in the cyclin D1b-overexpressing group (**Figure 2B** and **2C**; $P < 0.01$), indicating that upregulation of cyclin D1b inhibits cervical cancer cell proliferation and colony formation.

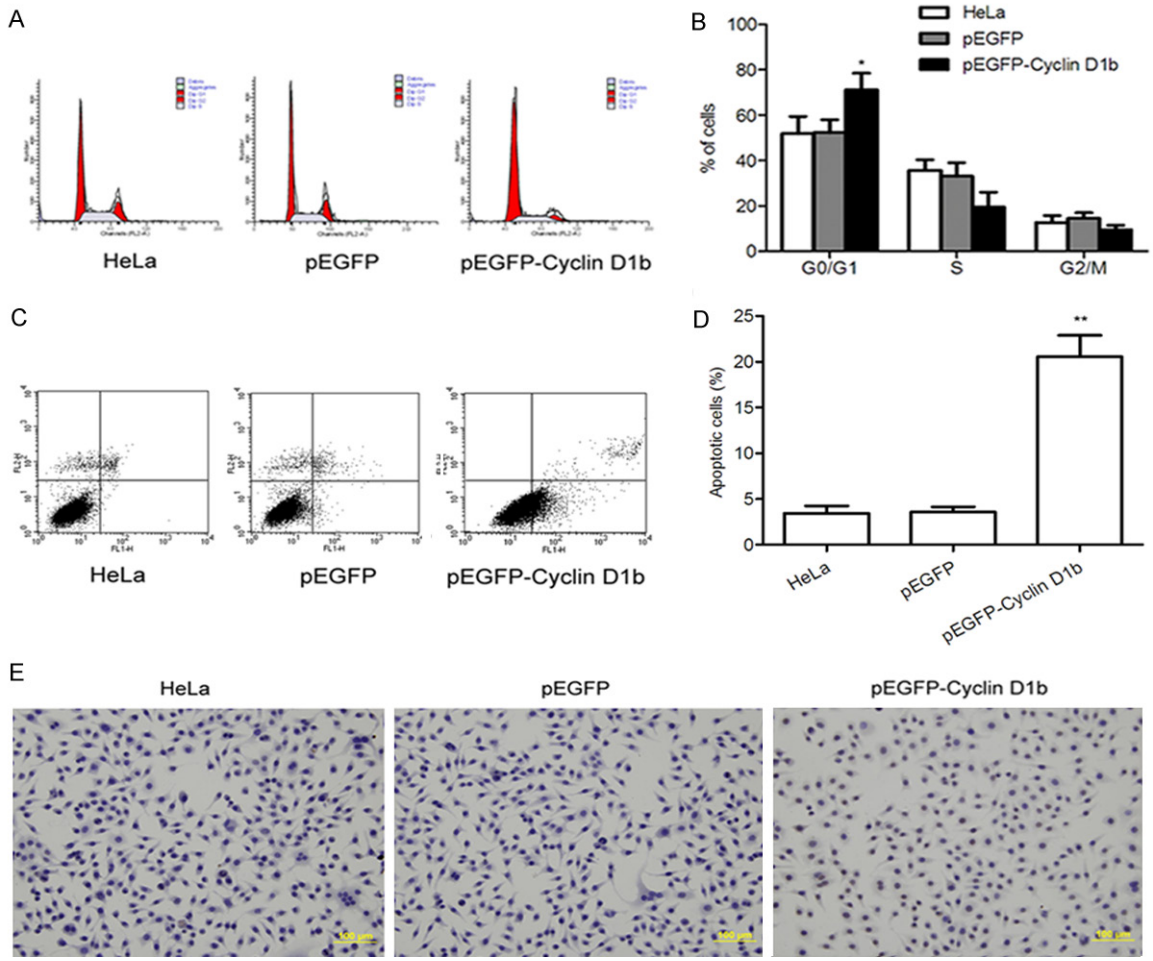

Figure 3. Upregulation of cyclin D1b induced cell cycle arrest and apoptosis. A. Flow cytometry was used to evaluate cell cycle arrest. Representative results obtained from three replicate experiments are presented in the figure. B. The proportion of cells in each cell cycle phase was assessed by flow cytometry, n = 3. C. Flow cytometry was used to detect cellular apoptosis. Representative results are presented in the figure. D. The proportion of apoptotic cells in each group was evaluated by flow cytometry, n = 3. E. The TUNEL assay was also used to detect apoptosis. Apoptotic nuclei are stained brown. Representative staining results for each group are presented in the figure; $*P < 0.05$, $**P < 0.01$, compared with the control group.

Upregulation of cyclin D1b induces cell cycle arrest and apoptosis in cervical cancer cells

To investigate the mechanisms underlying the inhibition of cell proliferation by cyclin D1b, we assessed changes in cell cycle progression after cyclin D1b overexpression using flow cytometry. Compared with the control group, the percentage of total cells at the G0/G1 phase was significantly increased in the cyclin D1b-overexpressing group (**Figure 3A** and **3B**; $P < 0.05$). As illustrated in Figure 3C and D, the results from apoptosis assays further demonstrated that the percentage of apoptotic cells in Cyclin D1b-overexpressing cells was significantly higher than that in the control group (20.54% ± 2.32% vs. 3.43% ± 0.82%, $P < 0.01$). In addition, we performed TUNEL assays to further evaluate cell apoptosis (**Figure 3E**). The nuclei were stained blue-purple in pEGFP-C1-transfected cells and control cells, whereas they displayed a remarkable brown color in pEGFP-Cyclin D1b-transfected cells. These data indicate that upregulation of Cyclin D1b arrests these cells in the G0/G1 phase of the cell cycle and induces cell apoptosis.

Upregulation of cyclin D1b inhibits the growth of xenograft tumors and induce apoptosis

Cells were transplanted subcutaneously into nude mice, and after tumor formation, the

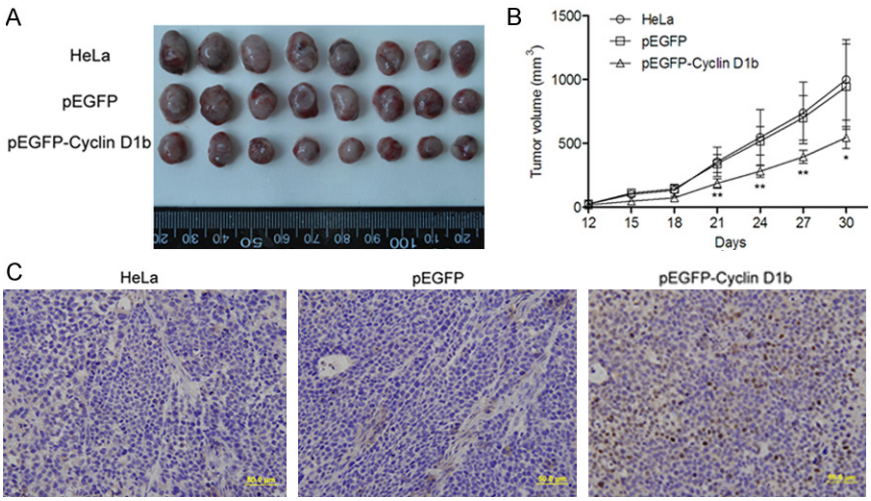

Figure 4. Upregulation of cyclin D1b inhibited the proliferation of cervical cancer cells and induced cell apoptosis in vivo. A. A total of 1×10^7 cells were seeded into the right forelimb armpit of nude mice, and 30 days after transplantation, the tumor tissues were removed for analysis. B. Tumor volume changes after xenograft transplantation in each group. The tumor volume was measured every 3 days after post-transplantation day 12, n = 8; C.The TUNEL assay was used to detect tumor cell apoptosis. Apoptotic nuclei are stained brown. Representative staining results for each group are presented in the figure; *P < 0.05, **P < 0.01, compared with the control group.

tumor volume was measured every 3 days to evaluate the effect of cyclin D1b on tumor growth in vivo. Compared with controls, the transfection of pEGFP-cyclin D1b showed significant inhibition on tumor growth (**Figure 4A** and **4B**; *P* < 0.05). Mice were sacrificed 30 days after transplantation, and the tumors were removed for analysis. As observed with the naked eye, the mice in the pEGFP-cyclin D1b-transfected group showed significantly small tumors. Furthermore, the assessment of tumor cell apoptosis in vivo demonstrated that the number of apoptotic cells with brown-stained nuclei in the cyclin D1b-overexpressing group was significantly increased compared to the other two groups (**Figure 4C**). Therefore, overexpression of cyclin D1b also inhibits tumor growth and induces apoptosis in vivo.

Discussion

Previous studies have revealed that cyclin D1b is involved in cell cycle regulation and transcriptional regulation, thus playing a pivotal role in tumor occurrence and progression [10, 12]. However, its definitive role in cervical cancer has not yet been reported. In this study, we established HeLa cells that stably overexpressed cyclin D1b and found that upregulation of cyclin D1b inhibited cervical cancer cell proliferation and colony formtion, arrested the cell cycle at the G0/G1 phase and induced cancer cell apoptosis. The xenograft transplantation expement further showed that upregulation of cyclin D1b inhibited the growth of cervical cancer cells and induced apoptosis in vivo. Therefore, cyclin D1b plays an important role in the pathogenesis and development of cervical cancer, and upregulation of cyclin D1b expresion represents a promising strategy for cervical cancer treatment.

Cyclin D1a and cyclin D1b are two transcripts produced by differential splicing of the polymorphic cyclin D1 gene. Initially, cyclin D1b was considered a nuclear proto-oncogene independent of cyclin D1a [15], and a number of studies have indicated that abnormally high expression of cyclin D1b is associated with poor prognosis in various cancers, such as breast cancer [16, 17], non-small cell lung cancer [18] and colon cancer in women [19]. However, studies using the T47D and MCF7 breast cancer cell lines suggest that cyclin D1b antagonizes the effect of cyclin D1a and inhibits the growth of breast cancer cells [12]. Our previous study found that the expression of cyclin D1b was downregulated in cervical cancer tissues, and the cyclin D1b expression levels were significantly lower in the patients with moderately and poorly differentiated cancers than those with highly differentiated cervical cancer [14]. In this study, both in vitro and in vivo results showed that upregulation of cyclin D1b inhibited cancer cell proliferation and induced apoptosis, indicating that upregulation of cyclin D1b may prevent the progression of cervical cancer. Therefore, it is possible that cyclin D1b may play different roles in different tissues or cells.

Cyclin D1a can bind to and activate the cyclin-dependent kinases CDK4 and CDK6, thus

mediating the phosphorylation of retinoblastoma (Rb) protein and inducing cell transition from the G1 to S phase [20]. Abnormal regulation of this process is closely associated with the occurrence and development of various tumors [21]. Compared with cyclin D1a, the C-terminus of cyclin D1b lacks the "PEST" sequence and residues that control nuclear export and protein stability. Previous investigations have also shown that compared with cyclin D1a, cyclin D1b has a significantly weaker impact on the phosphorylation of Rb, and its role in the malignant transformation of cells also differs from that of cyclin D1a [22]. Moreover, studies on B lymphocytes have demonstrated that cyclin D1a and cyclin D1b possess different biological functions, as cyclin D1b does not exert a regulatory effect on progression of the cell cycle [23]. In this study, upregulation of cyclin D1b arrested the cell cycle at the G0/G1 phase and significantly increased the proportion of apoptotic cells, indicating that the regulatory role of cyclin D1b in the cervical cancer cell cycle may also differ from that of cyclin D1a.

Persistent HPV infection is one of the major contributing factors to the development of cervical cancer [24]. After HPV DNA integrates into the host chromosome, the early oncoproteins E6 and E7 are expressed, which induces the activation of proto-oncogenes and the inactivation of tumor suppressor genes in host cells, eventually leading to the abnormal proliferation and malignant transformation of normal cervix epithelial cells [25-27]. Rb is one of the most important binding targets during E6 and E7-induced cell proliferation [28], and cyclin D1 and E7 show homology at Rb binding sites [29]. Therefore, upregulation of cyclin D1b may competitively inhibit the binding of E7 protein with Rb in cervical cancer, further hindering the gene expression required for cell replication and viral synthesis and inhibiting cell proliferation. However, the precise mechanism underlying the role of cyclin D1b in cervical cancer requires further exploration.

In summary, the results of this study indicate that upregulation of cyclin D1b inhibited cervical cancer cell proliferation and colony formation, arrested the cell cycle at the G0/G1 phase, and ultimately induced cell apoptosis. In addition, our in vivo experiments verified that upregulation of cyclin D1 inhibited the growth of cancer xenograft tumors in nude mice and induced apoptosis. Thus, cyclin D1b may represent a potential candidate for gene therapy in cervical cancer.

Acknowledgements

This study was supported by grants from the National Nature Science Foundation of China (No.: 81202047), the Program for Liaoning Excellent Talents in University (No: LJQ20-13083), the Higher Specialized Research Fund for the Doctoral Program (No: 2012210411-0014), and the Free Researcher Project of Shengjing Hospital (No: 201302).

Disclosure of conflict of interest

None.

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References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-2917.

[3] Shi JF, Canfell K, Lew JB and Qiao YL. The burden of cervical cancer in China: synthesis of the evidence. Int J Cancer 2012; 130: 641-652.

[4] Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ and Meijer CJ. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348: 518-527.

[5] Glick SB, Clarke AR, Blanchard A and Whitaker AK. Cervical cancer screening, diagnosis and treatment interventions for racial and ethnic minorities: a systematic review. J Gen Intern Med 2012; 27: 1016-1032.

[6] Bonilla C, Lefevre JH, Winney B, Johnstone E, Tonks S, Colas C, Day T, Hutnik K, Boumertit A, Midgley R, Kerr D, Parc Y and Bodmer WF. Cyclin D1 rare variants in UK multiple adenoma and early-onset colorectal cancer patients. J Hum Genet 2011; 56: 58-63.

[7] Hayakawa Y, Hirata Y, Nakagawa H, Sakamoto K, Hikiba Y, Kinoshita H, Nakata W, Takahashi R, Tateishi K, Tada M, Akanuma M, Yoshida H,

Takeda K, Ichijo H, Omata M, Maeda S and Koike K. Apoptosis signal-regulating kinase 1 and cyclin D1 compose a positive feedback loop contributing to tumor growth in gastric cancer. Proc Natl Acad Sci U S A 2011; 108: 780-785.

[8] Jirawatnotai S, Hu Y, Michowski W, Elias JE, Becks L, Bienvenu F, Zagozdzon A, Goswami T, Wang YE, Clark AB, Kunkel TA, van Harn T, Xia B, Correll M, Quackenbush J, Livingston DM, Gygi SP and Sicinski P. A function for cyclin D1 in DNA repair uncovered by protein interactome analyses in human cancers. Nature 2011; 474: 230-234.

[9] Betticher DC, Thatcher N, Altermatt HJ, Hoban P, Ryder WD and Heighway J. Alternate splicing produces a novel cyclin D1 transcript. Oncogene 1995; 11: 1005-1011.

[10] Burd CJ, Petre CE, Morey LM, Wang Y, Revelo MP, Haiman CA, Lu S, Fenoglio-Preiser CM, Li J, Knudsen ES, Wong J and Knudsen KE. Cyclin D1b variant influences prostate cancer growth through aberrant androgen receptor regulation. Proc Natl Acad Sci U S A 2006; 103: 2190-2195.

[11] Kim CJ, Nishi K, Isono T, Okuyama Y, Tambe Y, Okada Y and Inoue H. Cyclin D1b variant promotes cell invasiveness independent of binding to CDK4 in human bladder cancer cells. Mol Carcinog 2009; 48: 953-964.

[12] Zhu J, Sen S, Wei C and Frazier ML. Cyclin D1b represses breast cancer cell growth by antagonizing the action of cyclin D1a on estrogen receptor alpha-mediated transcription. Int J Oncol 2010; 36: 39-48.

[13] Krieger S, Gauduchon J, Roussel M, Troussard X and Sola B. Relevance of cyclin D1b expression and CCND1 polymorphism in the pathogenesis of multiple myeloma and mantle cell lymphoma. BMC Cancer 2006; 6: 238.

[14] Wang N, Qian X, Wang S, Gao H, Wang L, Huo Y and Zhang S. CCND1 rs9344 polymorphisms are associated with the genetic susceptibility to cervical cancer in Chinese population. Mol Carcinog 2012; 51: 196-205.

[15] Lu F, Gladden AB and Diehl JA. An alternatively spliced cyclin D1 isoform, cyclin D1b, is a nuclear oncogene. Cancer Res 2003; 63: 7056-7061.

[16] Abramson VG, Troxel AB, Feldman M, Mies C, Wang Y, Sherman L, McNally S, Diehl A and Demichele A. Cyclin D1b in human breast carcinoma and coexpression with cyclin D1a is associated with poor outcome. Anticancer Res 2010; 30: 1279-1285.

[17] Millar EK, Dean JL, McNeil CM, O'Toole SA, Henshall SM, Tran T, Lin J, Quong A, Comstock CE, Witkiewicz A, Musgrove EA, Rui H, Lemarchand L, Setiawan VW, Haiman CA, Knudsen KE, Sutherland RL and Knudsen ES. Cyclin D1b protein expression in breast cancer is independent of cyclin D1a and associated with poor disease outcome. Oncogene 2009; 28: 1812-1820.

[18] Li R, An SJ, Chen ZH, Zhang GC, Zhu JQ, Nie Q, Xie Z, Guo AL, Mok TS and Wu YL. Expression of cyclin D1 splice variants is differentially associated with outcome in non-small cell lung cancer patients. Hum Pathol 2008; 39: 1792-1801.

[19] Kim CJ, Tambe Y, Mukaisho K, Sugihara H, Isono T, Sonoda H, Shimizu T, Kondoh G and Inoue H. Female-specific rectal carcinogenesis in cyclin D1b transgenic mice. Carcinogenesis 2014; 35: 227-236.

[20] Coqueret O. Linking cyclins to transcriptional control. Gene 2002; 299: 35-55.

[21] Kim JK and Diehl JA. Nuclear cyclin D1: an oncogenic driver in human cancer. J Cell Physiol 2009; 220: 292-296.

[22] Solomon DA, Wang Y, Fox SR, Lambeck TC, Giesting S, Lan Z, Senderowicz AM, Conti CJ and Knudsen ES. Cyclin D1 splice variants. Differential effects on localization, RB phosphorylation, and cellular transformation. J Biol Chem 2003; 278: 30339-30347.

[23] Leveque C, Marsaud V, Renoir JM and Sola B. Alternative cyclin D1 forms a and b have different biological functions in the cell cycle of B lymphocytes. Exp Cell Res 2007; 313: 2719-2729.

[24] Schiffman M and Castle PE. Human papillomavirus: epidemiology and public health. Arch Pathol Lab Med 2003; 127: 930-934.

[25] Narisawa-Saito M and Kiyono T. Basic mechanisms of high-risk human papillomavirus-induced carcinogenesis: roles of E6 and E7 proteins. Cancer Sci 2007; 98: 1505-1511.

[26] Moody CA and Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. Nat Rev Cancer 2010; 10: 550-560.

[27] Gao P and Zheng J. High-risk HPV E5-induced cell fusion: a critical initiating event in the early stage of HPV-associated cervical cancer. Virol J 2010; 7: 238.

[28] Mighty KK and Laimins LA. The role of human papillomaviruses in oncogenesis. Recent Results Cancer Res 2014; 193: 135-148.

[29] Cho NH, Kim YT and Kim JW. Alteration of cell cycle in cervical tumor associated with human papillomavirus: cyclin-dependent kinase inhibitors. Yonsei Med J 2002; 43: 722-728.