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Original Article

Impacts of CyhospitalclinE downstream vimentin on proliferation, invasion and apoptosis of hepatoma HepG2 cell

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Abstract: Objective: To build vimentin (VIM) interference RNA eukaryotic expression vector and observe hepatoma HepG2 cell's capacity of proliferation, apoptosis, changes of its capability of invasion and transfer after CyclinE downstream VIM expression is suppressed. Methods: Firstly, CyclinE gene was knocked out by RNA inference; the changes of HepG2 cell's capacity of proliferation and level of VIM mRNA and protein were observed. Secondly, eukaryotic expression vector of CyclinE downstream VIM interference RNA was built. Thirdly, MTT and Transwell small chamber were used to detect the change of cell's capacity of proliferation and invasion after VIM was silent, and flow cytometry in level of apoptosis. Results: Eukaryotic expression vectors of new biological target VIM interference RNA were built, namely P^{VIM-1}, P^{VIM-2}, P^{VIM-3} and P^{VIM-4}. CyclinE gene knock-out could prominently suppress HepG2 cell proliferation. Level of VIM mRNA and protein expression was down-regulated after CyclinE-1mRNA was down-regulated. After VIM expression was down-regulated, HepG2 cell's capacity of proliferation and invasion were weakened and apoptosis increased. Conclusion: VIM is involved in proliferation, invasion, leading to more severe cell apoptosis.

Keywords: Interference RNA, CyclinE, vimentin, apoptosis

Introduction

Hepatoma is a malignant tumor with a high worldwide morbidity and high death rate, and require complex treatment [1]. Hepatoma has good capacity of invasion and transfer, and is apt to recur after operation. It is not sensitive to radiotherapy and chemotherapy, and often accompanied by unfavorable prognosis [2, 3]. Pathogenesis is not clear yet and there is no effective early diagnosis method and biological target for treatment. Current study shows that proto-oncogene activation and unbalanced cyclin regulation exist in hepatoma cell in comparison with normal liver cell [4]. As a key cyclin, CyclinE can promote pRB phosphorylation and combine with cyclin-dependent kinase inhibitor to turn cell cycle from phase G1 to S. Over-expression of CyclinE can shorten phase G1, advance the time for cell to enter phase S, impact replication and division of DNA and centrosome and interfere replication and division of cell nucleic acid, giving rise to instable gene expression and occurrence of tumor [5]. After making CyclinE silent by interfering RNA, this research group found that protein knock-out could significantly reduce invasion capacity of tumor cell and inhibit hepatoma cell proliferation [6, 7]. This shows CyclinE has important impacts on proliferation, invasion and transfer of tumor and has the potential to become a new biological target for tumor treatment. However, mechanism of action for downstream signal where CyclinE protein impacts hepatoma cell proliferation is not clear yet.

Vimentin (VIM) belongs to intermediate filament fibrin of type III. It is an important component of skelemin and plays a key role in maintaining cell integrity. It is indicated in research of recent years that VIM has certain regulation effect for several tumor cells with high expression, such as breast cancer, gastroenteric tumor, and etc. and tumor cell in processes of migration, adhesion, epithelial-mesenchymal transition, etc. [8, 9]. It is found in proteomics

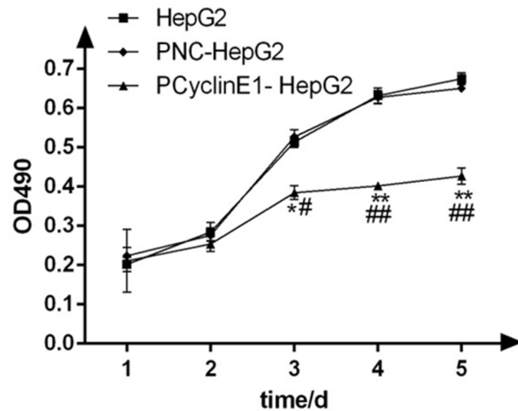

Figure 1. Effect of cycloneE gene knockdown on HepG2 cell growth. *P<0.05, compared with HepG2 group; **P<0.01, compared with HepG2 group; #P<0.05, compared with P^NC^-HepG2 group; ##P<0.01, compared with P^CyclinE^-HepG2 group. NC, negative control.

analysis of hepatoma carcinoma cell with CyclinE protein being knocked down in the early stage that silence of CyclinE has important influences on expression level of VIM, thus VIM protein is taken as entry point based on research basis in the early stage so as to investigate influences of CyclinE protein on relevant mechanisms of hepatoma carcinoma cell proliferation, invasion, and apoptosis through VIM protein.

Materials and methods

Materials

Hepatoma cell line HepG2 was purchased from Shanghai Cell Bank of Chinese Academy of Sciences with fetal calf serum from Sigma, DNA incision enzyme and DNA ligase from MBI, Lipofectamine 2000 transfection kit from Shanghai Yingjun Company, DNA gel extraction kit, and agarose. from Tiangen Biotech Co., Ltd., Transwell chamber from Corning, Annexin V-FITC/PI staining kit from Sigma, and CyclinE antibody (Art. No.10902-RP02-50), VIM antibody (Art. No.100254-T40-50), and GAPDH antibody (Art. No.100242-T40-50) from Beijing Sino Biological Inc.

Description for abbreviation of all transfection plasmids

Plasmid group which was not subject to transfection in the experiment was classified as HepG2; negative control (NC) plasmid group which was subject to transfection-P^{NC}-HepG2 [6]; $P^{CyclinE-1}$ plasmid group which was subject to transfection-$P^{CyclinE-1}$-HepG2; P^{VIM-1} plasmid group which was subject to transfection-P^{VIM-1}-HepG2; P^{VIM-2} plasmid group which was subject to transfection-P^{VIM-2}-HepG2; P^{VIM-3} plasmid group which was subject to transfection-P^{VIM-3}-HepG2; P^{VIM-4} plasmid group which was subject to transfection-P^{VIM-4}-HepG2.

Three experimental groups, respectively HepG2, P^{NC}-HepG2, and $P^{CyclinE-1}$-HepG2, were established according to influences of cycline-E1 gene knock-down on cell proliferation and lower VIM expression. Six experimental groups, respectively HepG2, P^{NC}-HepG2, P^{VIM-1}-HepG2, P^{VIM-2}-HepG2, P^{VIM-3}-HepG2, and P^{VIM-4}-HepG2, were established according to VIM gene knock-down experiment. Three experimental groups, respectively HepG2, P^{NC}-HepG2, and P^{VIM-1}-HepG2 were established according to influences of VIM gene knock-down on cell proliferation, apoptosis, and migration.

Construction of interfering RNA of new target point VIM

Negative controlwas set for Four pairs of VIM-siRNA target sequences which are interfering parts are respectively P^{VIM-1}: 5'-CTCTCTGAGGCTGCCAACCGGA-3', P^{VIM-2}: 5'-CTGTGAAGTGGATGCCCTTAAA-3', P^{VIM-3}: 5'-GAACGCAAAGTGGAATCTTTG-3', and P^{VIM-4}: 5'-CGTCAGCAATATGAAAGTGTG-3' at the same time for sequencing and identification.

Transfection of interfering plasmid to HepG2 cell

HepG2 cell were inoculated to six-well plates one day before transfection so as to set cell convergence degree at 70% on the very transfection day. Lipofectamine 2000 transfection cells were used; transfection operation process were conducted according to instruction for use of reagent. Cells were digested and collected 48 h later for follow-up experiment after transfection.

mRNA extraction and detection for relative expression level

Cell was collected, and Trizol method was adopted to extract the total RNA of cell. Extraction procedure was conducted strictly according to kit instruction. PRC primer was

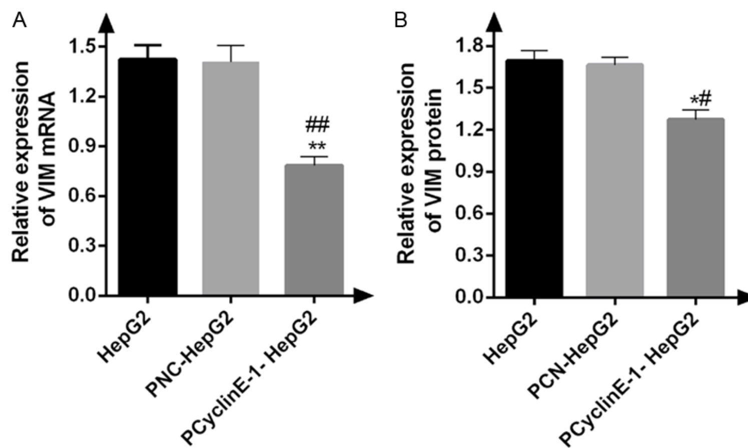

Figure 2. A. Effect of cyclinE gene knockdown on VIM mRNA expression in HepG2 cells; B. Effect of cyclinE gene knockdown on VIM protein expression in HepG2 cells. *P<0.05, compared with HepG2 group; **P<0.01, compared with HepG2 group; #P<0.05, compared with P^{NC}-HepG2 group; ##P<0.01, compared with $P^{CyclinE}$-HepG2 group. VIM, vimentin; NC, negative control.

designed aiming at target gene, and RT-PCR was adopted to detect expression level of target gene. Upstream and downstream primer of CyclinE were respectively: 5'-ACCAGTTT-GCGTATGTGAC-3'; 5'-CTGCTCTGCTTCTTACCG-3'. Upstream and downstream primer of VIM were respectively: 5'-GCGGTGACCTTGAACGC-AAAGTGGAA-3'; 5'-TGAGTTCTGTGCTCTCCTCG-CCTTCC-3'. Upstream and downstream primer of reference gene GAPDH were respectively 5'-CGGGAAACTGTGGCGTGAT-3'; 5'-GAGTGGGT-GTCGCTGTTGA-3'. PCR response procedure was as follows: initial denaturation of 3 min at 95°C, denaturation for 15 s at 95°C, annealing for 15 s at 62°C and extension for 30 s at 72°C, 35 circulations in total. After PCR response, identification for running the gel was conducted in 1.5% agarose gel and gel imaging system was adopted to analyze relative expression quantity of target gene. Relative expression quantity would be the ratio of average optical density value (IDV) for target gene and reference gene IDV. Jamming efficiency for all groups was calculated. Taking CyclinE gene as example, jamming efficiency for all groups = (1-(CyclinE mRNA relative expression quantity for per group)/(CyclinE mRNA relative expression quantity in HepG2 group)) * 100%.

Extraction of the total protein and western blot

The total protein was extracted from all groups of cells after transfection and BCA method was used to detect protein concentration. Sample of 50 μg protein was taken to conduct SDS-PAGE electrophoresis, then wet-turning method was adopted to transfer protein to PVDF film. TBST including 5% skim milk powder was sealed for 2 h, and target protein (CyclinE, VIM) and primary antibodies for b-actin GAPDH was placed at room temperature for incubation for 2 h. Dilution ratio was set at 1: 1,000, and dilution ratio of b-actin protein GAPDH 1:10,000. Secondary antibodies marked with horseradish peroxidase was incubated for 2 h at room temperature after TBST rinsing for 5 times, and it will develop after TBST rinsing for 5 times. Gray imaging software (UVP, UK) was adopted to measure optical of density value of main band to calculate expression level of the above protein in brain tissue.

Detecting multiplication capacity of cell through MTT method

Five ventral orifices were set per group, and one group was set as of blank control orifice. Cells were taken in all groups, and were inoculated in orifice plate with 4 * 10^3 cell concentration. Then, 20 μL 5 mg/mL MTT liquid was added into per orifice at 1-5 days. After cultivating for 4 hours, old liquid was removed, 200 μL DMSO was added, vibrating for 10 min. After that, microplate reader was used to detect 490 nm optical density, then it was calculated and conducted statistical analysis.

Detect cell invasion capability through Transwell test

Cells in exponential phase was digested, and cell density was re-suspended and adjusted to be 5 * 10^5/mL. Then, 500 μL cell suspension was added to upper chamber of Transwell chamber, and 500 mL DMEM complete medium was added to lower chamber. Three ventral orifices were set in per group of cell. Culture solution in the upper chamber was discarded and cotton swab was used to wipe off cell on filter membrane after placing cell on incubator for 12 h. Microscopic counting was conducted after migrated cell crystallizes into violet staining, and 5 different visions were selected for per orifice.

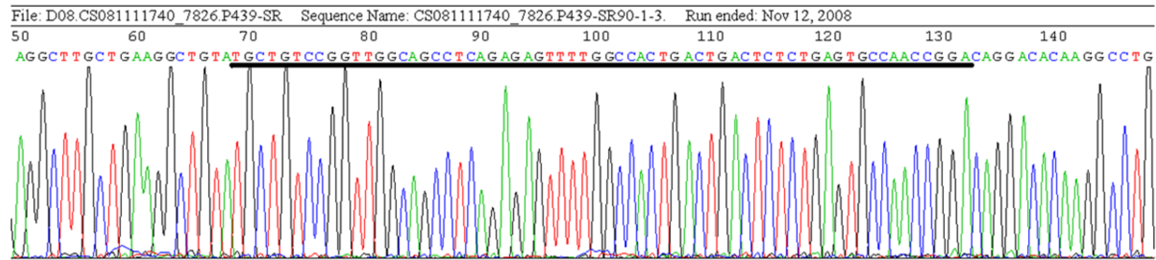

Figure 3. Partial sequencing diagram of P^{VIM-1} plasmid expression vector. VIM, vimentin.

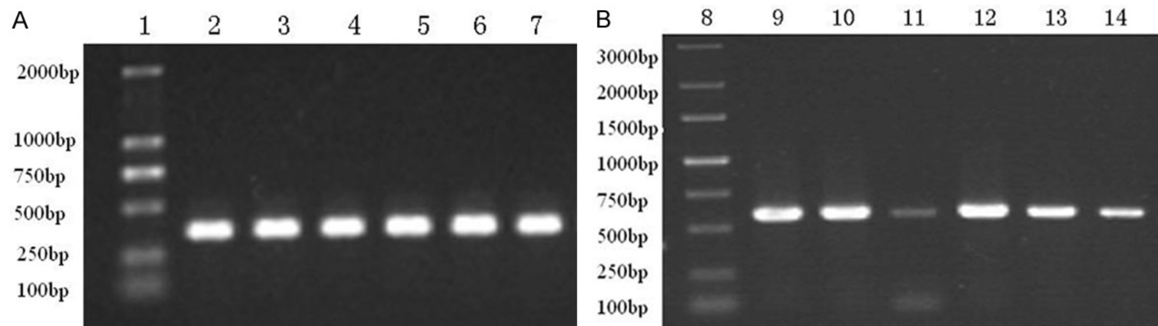

Figure 4. VIM mRNA RT-PCR detection of all groups. GAPDH PCR products of the normal HepG2, P^{NC}-HepG2, P^{VIM-1}-HepG2, P^{VIM-2}-HepG2, P^{VIM-3}-HepG2, P^{VIM-4}-HepG2 cells were added to the Lanes 1, 2, 3, 4, 5, 6 and 7 respectively; VIM PCR products of the normal HepG2, $P^{NC-siRNA}$-HepG2, P^{VIM-1}-HepG2, P^{VIM-2}-HepG2, P^{VIM-3}-HepG2 and P^{VIM-4}-HepG2 cells were added to Lanes 8, 9, 10, 11, 12, 13 and 14 respectively; VIM, vimentin; NC, negative control.

Detect cell apoptosis through flow cytometer

Cells in exponential phase was digested into cell suspension, and then was inoculated into 6 orifice plates for continuous culturing in 1.0 * 10^6/mL density. After culturing for 24 h, pancreatin was digested and manufactured into cell suspension. Then cell density was adjusted to 5 * 10^5/mL, and 4°C pre-cooling PBS (pH 7.4) was added for rinsing for 3 times after respectively taking 2 mL cell suspension for centrifugation, centrifuge at 1,000 rpm for 5 min. Cell was re-suspended into 100 μL blinding buffer, successively 5 μL annexin V-fluorescein isothiocyanate (PI) and 5 μL propidium Iodide (FITC) were added, slightly shocking and blending. A total of 400 μL blinding buffer was added after placing at room temperature for 15 min keeping out of the sun, immediately it was conducted detection on machine, and cell apoptosis rate was calculated in CellQuest analysis software.

Statistical analysis

The quantitative data in this study were all expressed as mean ± standard deviation ($\overline{x}$ ± sd); the mRNA and protein expression levels of the target genes, and the between-group differences of the apoptotic experiment, the Transwell chamber invasion experiment were adopted two-sample t-test. $P<0.05$ was was regarded as the test standard, and all data from statistical analysis shall be realized by using SPSS 19.0 software.

Results

Effect of CyclinE interfering RNA on HepG2 cell growth

Cells in $P^{CyclinE-1}$-HepG2 group grew slowly from the third day, which was significantly lower than that of P^{NC}-HepG2 cells and HepG2 cells. However, the growth rates of P^{NC}-HepG2 cells and HepG2 cells were not significantly different, as shown in **Figure 1**.

Effect of CyclinE gene knockdown on VIM mRNA and protein expression in HepG2 cells

The previously-constructed $P^{CyclinE-1}$ plasmid and transfected HepG2 were adopted-GAPDH as internal reference-to test the expression level of VIM mRNA and its protein. The results

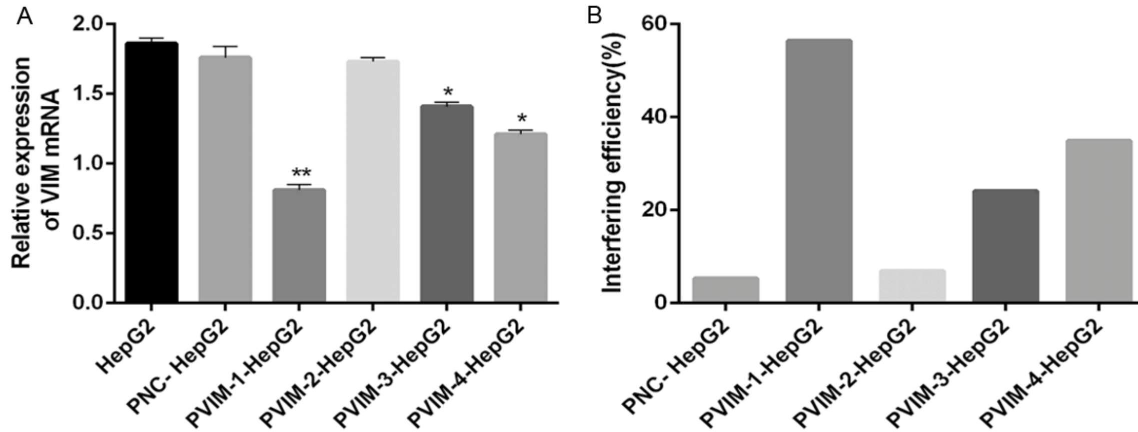

Figure 5. Effect of VIM silencing on VIM mRNA expression. A. Comparative analysis of VIM mRNA relative expression levels of different VIM interference plasmids; B. Comparative analysis of interference efficiency of different VIM interference plasmids. *P<0.05; **P<0.001; VIM, vimentin; NC, negative control.

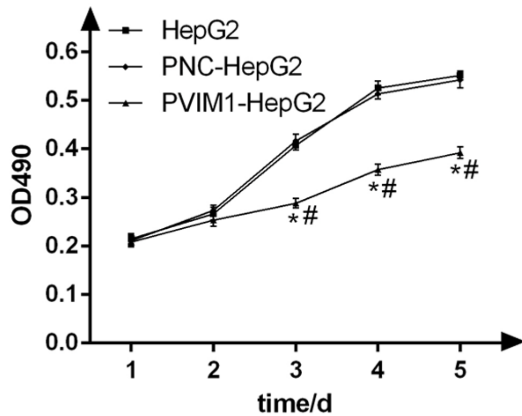

Figure 6. Effect of VIM gene knockdown on HepG2 cell proliferation. *P<0.01, compared with HepG2 group; #P<0.01, compared with P^{NC}-HepG2 group; VIM, vimentin; NC, negative control.

showed that the expression level of VIM mRNA and protein in $P^{CyclinE-1}$-HepG2 group were 0.783±0.057 and 1.272±0.068 respectively, that of PNC-HepG2 group were 1.407±0.101, 1.664±0.053 respectively and of HepG2 group were 1.422±0.087 and 1.694±0.071 respectively, if regarding GAPDH as the reference. The expression level of VIM mRNA and protein in $P^{CyclinE-1}$-HepG2 group was significantly lower than that in PNC-HepG2 group and HepG2 group, as shown in **Figure 2**.

VIM eukaryotic expression vector construction

Double enzyme digestion detection and base sequence determination showed that all the VIM eukaryotic expression vectors and negative control vectors were identical to the designed sequence, and no abnormality was found, as shown in **Figure 3**.

Effect of VIM silencing on VIM mRNA levels

The results show that the calculation method was using the same VIM, and the interference efficiency of each group was calculated, as shown in **Figures 4** and **5**. It was concluded that the interference efficiency of P^{VIM-1}-HepG2 group was the highest, up to 56%; therefore, all the subsequent experiments adopted this group as the experimental group.

Effect of VIM interfering RNA on HepG2 cell growth

Compared with the control cells, the cells in P^{VIM1}-HepG2 group grew slowly from the third day, and were significantly lower than the P^{NC}-HepG2 and HepG2 cells. However, the growth rates of P^{NC}-HepG2 and HepG2 cells were not significantly different, as shown in **Figure 6**.

Effect of VIM interfering RNA on HepG2 cell apoptosis

The apoptosis level of all groups of cells were detected by flow cytometry and the results shoed that the apoptosis rate of P^{VIM-1}-HepG2 cells was 26.14±3.82, and that of HepG2 cells was 8.52±2.13, and of P^{NC}-HepG2 cells was 13.03±2.66. The apoptosis rate of P^{VIM-1}-HepG2 cells was significantly higher than that of Hep-

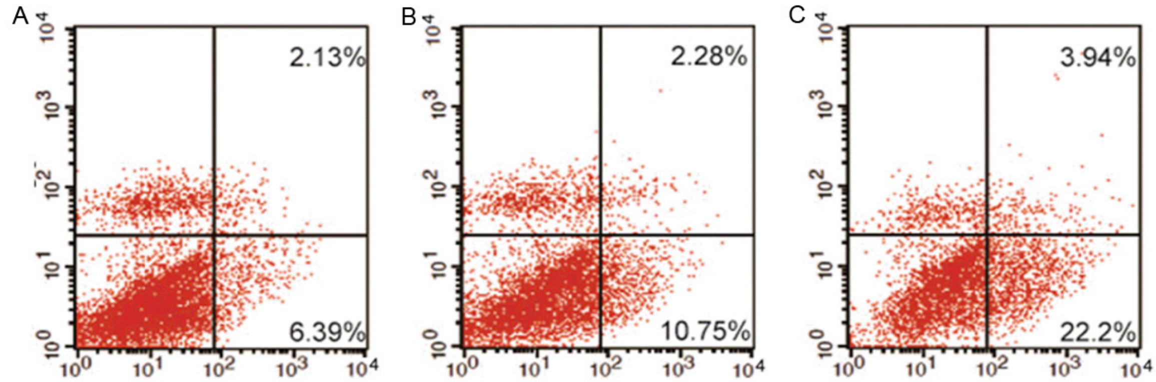

Figure 7. Effect of VIM interfering RNA on HepG2 cell apoptosis. A. HepG2 group; B. P^{NC}-HepG2 group; C. $P^{CyclinE-1}$-HepG2 group; VIM, vimentin; NC, negative control.

Table 1. Apoptosis rate of each group

Group	Apoptosis rate (mean ± sd, %)
HepG2 group	8.52±2.13
P^{NC}-HepG2 group	13.03±2.66
P^{VIM1}-HepG2 group	26.14±3.82$^{*,\#}$

Note: *P<0.05, compared with HepG2 group; #P<0.05, compared with PNC-HepG2 group; VIM, vimentin; NC, negative control.

Table 2. Numbers of transmembrane cells of all groups

Group	Number of invasive cells (mean ± sd, n)
HepG2 group	78.3±3.2
P^{NC}-HepG2 group	75.0±6.0
P^{VIM1}-HepG2 group	39.3±1.5$^{*,\#}$

Note: *P<0.05, compared with HepG2 group; #P<0.05, compared with P^{NC}-HepG2 group; VIM, vimentin; NC, negative control.

G2 group and P^{NC}-HepG2 group (P_1 = 0.0110, P_2 = 0.0240), as shown in **Figure 7** and **Table 1**.

Effect of VIM interfering RNA on invasion ability of HepG2 cells

Count the number of transmembrane cells in 12 hours under 200 magnified visual field, and the results showed that there were (39.3±1.5) in P^{VIM1}-HepG2 group, (78.3±3.2) in HepG2 group and (75.0±6.0) in P^{NC}-HepG2 group. The number of invasive cells in PVIM1-HepG2 group was significantly less than that in HepG2 group and PNC-HepG2 group (P_1 = 0.0021, P_2 = 0.0027), as shown in **Table 2**.

Discussion

Because cyclin can influence important role of cell proliferation through regulating cell cycle, it is used as an important target spot for tumor therapy at present. It can be researched and shown through method of RNA interference and inhibitor block in many researches that restraining expression of cyclin can restrain proliferation, migration, and transfer of cancer cell [10, 11]. CyclinE consists of 395 amino acids and it will take effect on G1 later period, however, the exertion of its function can depend on CyclinE-Cyclindependent kinase2 (CDK2) compound formed with combination of CDK2 to regulate phosphorylation process of G1 phase and S phase of cell. Our research result reveals that proliferation of HepG2 hepatoma cell can be reduced significantly through silencing CyclinE with RNA interference and it indicates there is important regulatory role of CyclinE to proliferation of hepatoma cell. Research of Liu et al. also shows doen-regulation of CyclinE can restrain proliferation and immigration of lymphoma cell [12].

VIM is an important biomarker of Epithelial-mesenchymal transition (EMT) for tumour and it can influence biological function of many tumor cells. Especially, it plays an important role in invasion and transfer of tumor cancel [13, 14]. Earlier research shows that cyclin participating in generation and development of EMT has certain influence on expression of VIM, at the same time, phosphorylation of VIM protein can influence regulation of cyclin protein to cancel cycle [15, 16]. We have detected in the research that VIM mRNA and expression

situation of protein after knock CyclinE out. The result shows that CyclinE knockout can restrain Vimentin mRNA and expression of protein, while silence of VIM can reduce invasion and migration capability of HepG2 cancer, increase apoptosis level of HepG2 cell, and hint that CyclinE can mediate proliferation, invasion, and immigration of HepG2 cell through VIM.

In VIM cell, 14-3-3 regulatory protein can be combined and activity of 14-3-3 protein that can regulate signal molecule can boost release of apoptosis factor. Upon silence of VIM, restrain of 14-3-3 protein has been lost and it leads to increase of apoptosis [17]. However, reduction of expression of vimentin can active caspase3 and then it will be further hydrolyzed so as to lead agglutination of cell nucleus and generate nucleus fragment easily. No matter VIM is hydrolyzed by protease or there is autologous depolymerization so that it forms small fragments and granulate polymer due to breakage, connection with cell nucleus vanishes totally or partly, and it leads to apoptosis [18]. Research also shows that caspase4 after being activated by some factors can degrade vimentin and destroy compound formed by vimentin and p53 protein so as to induce cell apoptosis [19]. Besides, reduction of VIM will change cell structure without reaching force-bearing requirement of normal cell skeleton, and some biological function cannot be completed with damaging of skeleton and it may oppress and even destroy some organelles so as to induce cell apoptosis [20].

Although it explores that there is close relationship between HepG2 hepatoma cell CyclinE and VIM preliminarily in the Research. CyclinE can restrain proliferation of HepG2 hepatoma cell and VIM plays an important role in the process with certain limitation. First of all, related signal pathway and specific function mechanism of CyclinE for VIM is not explicit and it can be researched further. Besides, only related experiments for HepG2 hepatoma cell is verified in the Research and whether the atmosphere exists in other hepatoma cells needs to be further verified. Eventually, it is not explicit that if there is another regulatory protein participating in influence of CyclinE for proliferation of HepG2 hepatoma cell.

In conclusion, with RNA interference technology, RNA eukaryotic expression vector of new CyclinE interference is designed and constructed and it is transfected to HepG2 cell so that obvious reducing of proliferation capability is found after reducing of CyclinE expression to analyze regulatory of CyclinE for VIM expression. And new RNA eukaryotic expression vector of VIM interference has been continued to constructing with increased apoptosis and decreased invasion capability. It will provide more deep and detailed materials for function mechanism of VIM and CyclinE and hepatoma pathogenesis and experimental basis for early diagnosis and therapy of hepatoma.

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Disclosure of conflict of interest

None.

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References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
[2] Andreou A, Bahra M, Schmelzle M, Ollinger R, Sucher R, Sauer IM, Guel-Klein S, Struecker B, Eurich D, Klein F, Pascher A, Pratschke J and Seehofer D. Predictive factors for extrahepatic recurrence of hepatocellular carcinoma following liver transplantation. Clin Transplant 2016; 30: 819-827.
[3] Uchiyama H, Minagawa R, Itoh S, Kajiyama K, Harimoto N, Ikegami T, Yoshizumi T, Shirabe K,

Takenaka K and Maehara Y. Favorable outcomes of hepatectomy for ruptured hepatocellular carcinoma: retrospective analysis of primary R0-hepatectomized patients. Anticancer Res 2016; 36: 379-385.

[4] Phang CW, Karsani SA, Sethi G and Abd Malek SN. Flavokawain C inhibits cell cycle and promotes apoptosis, associated with endoplasmic reticulum stress and regulation of MAPKs and Akt signaling pathways in HCT 116 human colon carcinoma cells. PLoS One 2016; 11: e0148775.

[5] Pok S, Wen V, Shackel N, Alsop A, Pyakurel P, Fahrer A, Farrell GC and Teoh NC. Cyclin E facilitates dysplastic hepatocytes to bypass G1/S checkpoint in hepatocarcinogenesis. J Gastroenterol Hepatol 2013; 28: 1545-1554.

[6] Hu GJ, Gao NK and Han YQ. Effects of CyclinE RNA interference on the invasion ability of human brain glioma. China Medical Herald 2014; 11: 9-12.

[7] Wang WL and Liu T. The effects of sirna-cyclin e on cell proliferation and proteome in hepatocellular carcinoma hepg2 cell line. Acta Academiae Medicine Neimongol 2008; 30: 229-235.

[8] Kim BR, Jeon YK and Nam MJ. A mechanism of apigenin-induced apoptosis is potentially related to anti-angiogenesis and anti-migration in human hepatocellular carcinoma cells. Food Chem Toxicol 2011; 49: 1626-1632.

[9] Chernoivanenko IS, Minin AA and Minin AA. Role of vimentin in cell migration. Ontogenez 2013; 44: 186-202.

[10] Deng M, Wang J, Chen Y, Zhang L, Xie G, Liu Q, Zhang T, Yuan P and Liu D. Silencing cyclin-dependent kinase inhibitor 3 inhibits the migration of breast cancer cell lines. Mol Med Rep 2016; 14: 1523-1530.

[11] Chen Y, Rao X, Huang K, Jiang X, Wang H and Teng L. FH535 inhibits proliferation and motility of colon cancer cells by targeting wnt/beta-catenin signaling pathway. J Cancer 2017; 8: 3142-3153.

[12] Liu P and Chen L. Inhibition of sonic hedgehog signaling blocks cell migration and growth but induces apoptosis via suppression of FOXQ1 in natural killer/T-cell lymphoma. Leuk Res 2018; 64: 1-9.

[13] Zhu QS, Rosenblatt K, Huang KL, Lahat G, Brobey R, Bolshakov S, Nguyen T, Ding Z, Belousov R, Bill K, Luo X, Lazar A, Dicker A, Mills GB, Hung MC and Lev D. Vimentin is a novel AKT1 target mediating motility and invasion. Oncogene 2011; 30: 457-470.

[14] Vuoriluoto K, Haugen H, Kiviluoto S, Mpindi JP, Nevo J, Gjerdrum C, Tiron C, Lorens JB and Ivaska J. Vimentin regulates EMT induction by Slug and oncogenic H-Ras and migration by governing Axl expression in breast cancer. Oncogene 2011; 30: 1436-1448.

[15] Jiao J, Huang L, Ye F, Shi M, Cheng X, Wang X, Hu D, Xie X and Lu W. Cyclin D1 affects epithelial-mesenchymal transition in epithelial ovarian cancer stem cell-like cells. Onco Targets Ther 2013; 6: 667-677.

[16] Chiu HC, Huang WR, Liao TL, Wu HY, Munir M, Shih WL and Liu HJ. Suppression of vimentin phosphorylation by the Avian reovirus p17 through inhibition of CDK1 and Plk1 impacting the G2/M phase of the cell cycle. PLoS One 2016; 11: e0162356.

[17] Huo Y, Zheng Z, Chen Y, Wang Q, Zhang Z and Deng H. Downregulation of vimentin expression increased drug resistance in ovarian cancer cells. Oncotarget 2016; 7: 45876-45888.

[18] Byun Y, Chen F, Chang R, Trivedi M, Green KJ and Cryns VL. Caspase cleavage of vimentin disrupts intermediate filaments and promotes apoptosis. Cell Death Differ 2001; 8: 443-450.

[19] Yang X, Wang J, Liu C, Grizzle WE, Yu S, Zhang S, Barnes S, Koopman WJ, Mountz JD, Kimberly RP and Zhang HG. Cleavage of p53-vimentin complex enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis of rheumatoid arthritis synovial fibroblasts. Am J Pathol 2005; 167: 705-719.

[20] Lin M, Pan J, Chen Q, Xu Z, Lin X and Shi C. Overexpression of FOXA1 inhibits cell proliferation and EMT of human gastric cancer AGS cells. Gene 2018; 642: 145-151.