

Original Article

Impact of PKM2 gene silencing on biological behavior of HepG2 cells

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Abstract: Objective: To investigate the impact of down-regulating pyruvate kinase M2 (PKM2) on the proliferation and invasion of hepatocellular carcinoma (HCC) HepG2 cells. Methods: The expression of PKM2 proteins in HepG2 cells was detected by immunofluorescence. Liposomes were used to transfect HepG2 cells with PKM2-shRNA recombinant plasmids. The expression level of PKM2 mRNA and proteins were detected by real-time fluorescent quantitative PCR and Western blotting, respectively. On one hand, the impact of PKM2 gene silencing on the proliferation, apoptosis and invasion ability of HepG2 cells were detected by CCK-8 method, FCM method and Transwell invasion assay, respectively; on the other hand, its impact on the expression of proliferation-associated proteins c-myc and cyclin D1 and apoptosis-associated proteins Bcl-2 and Bax was detected by Western blotting. A subcutaneous tumor model was established in BAL B/C nude mice with HepG2, HepG2/NC and HepG2/PKM2-shRNA cells and was observed for tumor growth. Results: PKM2 expressed both in the cytoplasm and nucleus of HCC HepG2 cells. After HepG2 cells were transfected with PKM2-shRNA plasmids for 48 h, the expression level of PKM2 mRNA and proteins in HepG2 cells was down-regulated significantly ($P < 0.01$), the proliferation and invasion ability of HepG2 cells was inhibited evidently, and apoptosis was promoted ($P < 0.05$). Silencing PKM2 gene down-regulated the expression of proteins c-myc, cyclin D1 and Bcl-2 ($P < 0.05$) and up-regulated that of protein Bax ($P < 0.05$). Results of *in vivo* tumor growth assay showed that, after over-expression of PKM2, tumor growth in mice with HepG2/PKM2-shRNA cells slowed down significantly compared with those with HepG2 cells and HepG2/NC cells ($P < 0.01$). Conclusion: Through PKM2-shRNA interference with PKM2 expression, cell proliferation was inhibited by down-regulating the expression of c-myc and cyclin D1 and apoptosis was promoted by down-regulating the expression of Bcl-2 and up-regulating the expression of Bax. PKM2 gene silencing could inhibit the proliferation and invasion of HCC HepG2 cells.

Keywords: HCC, PKM2, RNA interference, cell proliferation, cell invasion, apoptosis

Introduction

Hepatocellular carcinoma (HCC) was the most common liver malignant tumor in adults. Although its treatment had been promoted continuously, including excision, liver transplantation and chemoradiotherapy, no significant improvement had been made in terms of the overall five-year survival rate in HCC patients [1, 2]. One important reason why HCC had high fatality rate and poor prognosis was that the molecular mechanism of HCC genesis and development was still poorly understood.

However, with the development of modern cell and molecular biology, it had been demonstrated that the formation of HCC was mostly accompanied with abnormalities of molecular

and signaling pathways. Therefore, it would open up a new way for the clinical prevention and treatment of HCC to explore HCC genesis and development at molecular level and take relevant molecules as anti-tumor targets.

Pyruvate kinase (PK) was one of them. As a key enzyme in glycolysis, PK could catalyze phosphoenolpyruvic acid to yield pyruvate and release energy [3]. PK had four subtypes: PKL, PKR, PKM1 and PKM2. It was noteworthy that PKM1 was widely expressed in adult tissues and PKM2 was highly expressed in embryonic and tumor tissues [3-5]. It was reported that, PKM2, a rate-limiting enzyme of glycometabolism, could also act as a transcriptional coactivator [6, 7]. After being transfected into nucleus, it took part in the formation of resistance

Impact of PKM2 gene silencing on biological behavior of HepG2 cells

[8], mitosis [9] and cell cycle regulation [10, 11]. Currently, the relation between PKM2 and cancer had been rarely reported and the specific mechanism was unknown. Whether PKM2 was associated with the genesis and development of HCC and what was its role in the progression of HCC? To answer these questions, this study took HCC cell line HepG2 with PKM2 positive expression as the object and investigated the impact of down-regulating PKM2 expression on the proliferation, invasion and apoptosis of HepG2 cells via shRNA interference with PKM2 expression. In this way, we expected to provide a new way for further studies on the pathogenesis of HCC and to explore new targets for the treatment of HCC.

Material and methods

Cells and reagents

Human HCC cell line HepG2 was purchased from ATCC; Trypsin, fetal calf serum and DMEM culture solution from Gibco (the US); Matrigel from Sigma (the US); TRIzol, reverse transcription reagents and reagents associated with real-time fluorescent quantitative PCR reaction from TaKaRa (Nippon); LipofectAMINE 2000 and primer from Invitrogen (the US); rabbit anti-human PKM2, c-myc, cyclin D1, Bcl-2 and Bax monoclonal antibodies from CST (the US); rabbit anti-human β -actin polyclonal antibodies from Bioworld (the US); Horseradish Peroxidase (HRP) or fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG from ZSGB-Bio (Beijing, China); kits for protein lysis and extraction and BCA protein quantification as well as reagents associated with Western blotting from Beyotime Institute of Biotechnology; Transwell with 8.0 μ m pore and PVDF membrane from Millipore (the US); CCK-8 from 7sea Biotech (Shanghai, China); Annexin V-PE/7-AAD apoptosis assays kit from BD (the US); Recombinant plasmid pGIPZ-NCshRNA constructed with negative control shRNA (NC-shRNA) and pGIPZ-PKM2-shRNA constructed with PKM2 shRNA targeting PKM2 gene specifically from China Peptides Co., Ltd. The sequence of NC-shRNA was 5'-GCTTCTAACACCGGAGGTCTT-3', while the sequence of PKM2-shRNA was 5'-CATCTACCACTGCAATTA-3' [12].

Cell culture

HepG2 cells were inoculated in DMEM culture solution with 10% fetal calf serum. The solution

was then cultured in a sterile constant incubator whose CO₂ volume fraction was 5% under 37°C. After digestion with 0.25% trypsin and normal passage, cells during the logarithmic phase of growth were taken for assay.

The expression of PKM2 proteins in HepG2 cells

HepG2 cells during the logarithmic phase of growth, after digestion, were inoculated in a 12-well plate with slides placed in advance. When cell confluence reached up to 90%, culture solution was discarded. 4% paraformaldehyde solution was used to fix cells for 20 min at room temperature, 0.25% Triton X-100 to permeabilize cells for 20 min and blocking buffer with 5% goat serum for 30 min. Rabbit anti-human PKM2 monoclonal antibodies diluted by blocking buffer (volume ratio of 1:100) was added for reaction at 4°C overnight. Then, FITC-labeled goat anti-rabbit IgG (secondary antibody, red fluorescence) (volume ratio of 1:100) was added and the solution was incubated away from light at 37°C for 1 h. Afterwards, DAPI staining was conducted in dark for 10 min (nuclear staining). Finally, after sealing with glycerin, the expression of PKM2 proteins was observed under a fluorescence microscope (magnified 400 times) and photographed. This detection was repeated for three times.

Real-time fluorescent quantitative PCR

HepG2 cells in the logarithmic phase of growth were inoculated in a 6-well plate. When cell confluence reached up to 70% to 80%, they were transfected with 1.5 μ g pGIPZ-NC-shRNA and 1.5 μ g pGIPZ-PKM2-shRNA by the way presented in the instruction for use of Lipofectamine 2000. There were three groups as follows in this assay: (1) Blank control with HepG2 cells not transfected with any plasmid and liposomes having the same volume as those in the negative control and PKM2-shRNA group; (2) Negative control with HepG2 cells transfected with recombinant plasmid pGIPZ-NCshRNA; (3) PKM2-shRNA group with HepG2 cells transfected with recombinant plasmid pGIPZ-PKM2-shRNA. 48 h after transfection, total RNA was extracted and cDNA was synthesized according to the instruction for use of TaKaRa reverse transcription kit. Afterwards, by using cDNA as a template, the expression level of PKM2 mRNA was detected by real-time fluorescent quantitative PCR. The upstream

Impact of PKM2 gene silencing on biological behavior of HepG2 cells

primer sequence of PKM2 gene was 5'-CC-ATCCTCTACCGGCCCGTTG-3', the downstream primer sequence 5'-CCAGCCACAGGATGTTCTC-GTC-3' and the amplified fragment length 174 bp. The upstream primer sequence of internal reference β -actin was 5'-CCTTCCTGGGCATGGAGTCCT-3', the downstream primer sequence 5'-GGAGCAATGATCTTGATCTTC-3' and the amplified fragment length 202 bp. Reaction conditions were as follows: pre-denaturation at 95°C for 30 s; 95°C for 20 s, 59.4°C for 30 s, 72°C for 20 s for 40 cycles; final extension at 72°C for 10 min. By taking β -actin as an internal reference, we used $2^{-\Delta\Delta Ct}$ to show the expression level of PKM2 mRNA. In each group, there were three wells and an average value was calculated. This detection was repeated for three times.

The effect of PKM2 gene silencing detected by western blotting

Grouping, cell inoculation and transfection methods were the same as those used in section 1.4. 48 h after transfection, total protein was extracted and protein concentration was detected by BCA method. In each group, 50 μ g proteins was loaded and separated on 10% SDSPAGE. The separated protein was then electrotransferred to a PCDF membrane (pore size: 0.45 μ m) and was sealed in TBST with 5% skim milk powder under 37°C for 2 h. After that, rabbit anti-human PKM2 and rabbit anti-human β -actin polyclonal antibody (with a dilution ratio of 1:1000) were added for reaction at 4°C overnight. Then, the HRP-labeled goat anti-rabbit IgG (secondary antibody) (with a dilution ratio of 1:1000) was added after membrane cleaning for reaction at room temperature for 1 h. Again, after cleaning, membrane development was performed. The gray value of target stripes was analyzed by using software Quantity One 4.6.2 and the relevant expression level of target proteins was presented as the ratio of this value of target stripes to that of the internal reference β -actin. This detection was repeated for three times.

CCK-8 assay

Grouping, cell inoculation and transfection methods were the same as those used in section 1.4. 48 h after transfection, cells were digested by trypsin and were then re-suspended to adjust cell density to 20000 cells/ml. In a 96-well plate, 100 μ L cell suspension was inoculated in each well and 5 wells were allocated

in each group. Then, the culture plate was placed in a cell incubator to be culture for 24, 36, 48, 60 and 72 h. At each time point, 10 μ L CCK-8 was added in each well in the selected group of cells which was cultured for another 1.5 h. Finally, the absorbance (D) value in each well was measured at the wavelength of 450 nm with a microplate reader and proliferation inhibition rate was calculated. This detection was repeated for three times.

Proliferation inhibition rate (%) = (D value in blank control - D value in shRNA transfection group)/D value in blank control \times 100%.

Apoptosis assay

Grouping, cell inoculation and transfection methods were the same as those used in section 1.4. 48 h after transfection, cells were digested by trypsin without EDTA, centrifuged and collected. These cells were then washed with PBS twice, collected again and stored at low temperature. Afterwards, they were treated by an Annexin V-PE/7-AAD apoptosis detection kit according to its operating instructions. Cells labeled by Annexin V-PE and 7-AAD were loaded in a flow cytometry (Model: Becton Dickinson) to detect apoptosis rate. This detection was repeated for three times.

Transwell invasion assay

Grouping, cell inoculation and transfection methods were the same as those used in section 1.4. Matrigel was diluted by DMEM culture solution without serum with a volume ratio of 1:8. Transwell was placed into a 24-well plate. 50 μ L diluted Matrigel was added in upper wells and stood in an incubator at 37°C for 30 min. After that, the supernate of Matrigel was removed. Cells, 48 h after transfection, were digested, centrifuged, and re-suspended by DMEM culture solution without serum to adjust cell density to 1×10^5 cells/ml. Then, 200 μ L cell suspension was added into upper wells, while 600 μ L culture solution with 15% fetal calf serum was added into lower ones. The plate was then cultured in a sterile constant incubator whose CO₂ volume fraction was 5% under 37°C for 24 h. When taken out, it was washed with PBS for three times and cells which did not invade or metastasize were wiped off with a cotton swab. After the plate was dried in the air, crystal violet was added for staining for 10 min. Afterwards, the plate was washed

Impact of PKM2 gene silencing on biological behavior of HepG2 cells

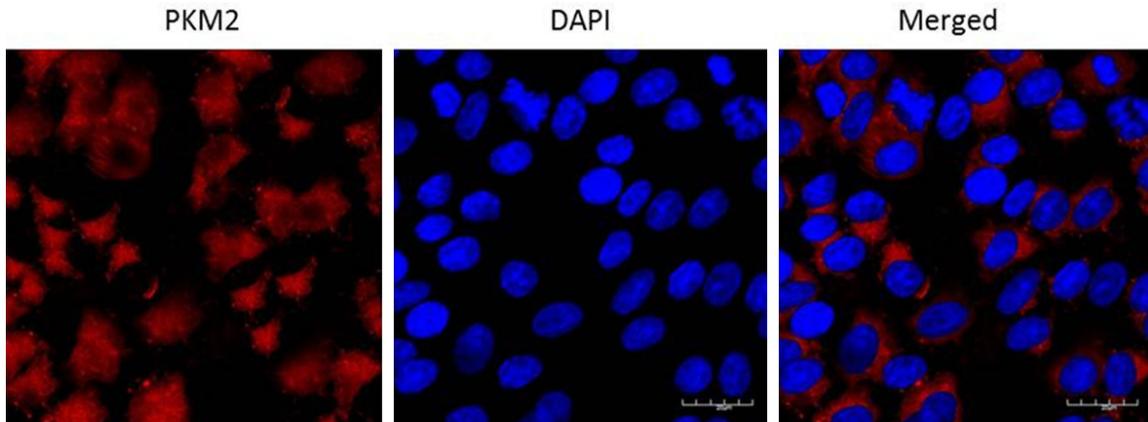


Figure 1. The expression of pyruvate kinase M2 (PKM2) protein in HepG2 cells was detected by immunofluorescence assay (observed under a fluorescence microscope, $\times 400$).

with PBS for three times, dried in the air, and observed for cells attached to the bottom of filter membrane under optical microscope. In each group, five fields of vision (magnified 200 times) were selected at random and counted for the number of cells which reflected their invasion ability. This detection was repeated for three times.

The expression of c-myc, cyclin D1, Bcl-2 and Bax

Grouping, cell inoculation, transfection methods and protein detection were the same as those used in section 1.5. Primary antibodies were c-myc, cyclin D1, Bcl-2 and Bax monoclonal antibody (the dilution ratio was all 1:10000; β -actin was also used as an internal reference) and secondary antibodies was HRP-labeled goat anti-rabbit IgG (the dilution ratio was 1:3000). The gray value of target stripes was analyzed by using software Quantity One 4.6.2 and the relevant expression level of target proteins was presented as the ratio of this value of target stripes to that of the internal reference β -actin. This detection was repeated for three times.

In vivo anti-tumor experiment

Female BAL B/C nude mice at 6-8 weeks of age were inoculated subcutaneously with 5×10^6 HepG2, HepG2/NC and HepG2/ PKM2-shRNA cells. Then, the length and width of tumor in mice was measured every week and tumor formation and growth were calculated. Tumor volume was calculated according to the following formula:

$$\text{Tumor volume} = 1/2 \text{ length} \times \text{width}^2$$

Six weeks later, mice were killed by cervical dislocation. Tumor tissues were weighed and statistical analysis was conducted for this weight.

Statistical methods

Statistical analysis of all experimental data was conducted by using software SPSS 17.0. Measurement data was expressed as $X \pm s$. Comparison among groups was made by one-way analysis of variance and comparison within group was made by LSD-t method. $P < 0.05$ meant that there was statistically significant difference.

Results

The expression of PKM2 in HepG2 cells

Results of immunofluorescence assay (**Figure 1**) showed that PKM2 proteins (labeled by red fluorescence) distributed both in cytoplasm and nucleus (shown as blue fluorescence after DAPI staining). It demonstrated that PKM2 was widely expressed in the cytoplasm and nucleus of HCC HepG2 cells. It provided a basis for down-regulating PKM2 expression in HepG2 cells by transfecting with recombinant plasmid PKM2 shRNA.

The expression of PKM2 mRNA and proteins in HepG2 cells transfected with PKM2-shRNA

Real-time fluorescent quantitative PCR test (**Figure 2A**) indicated that the expression level of PKM2 mRNA in PKM2-shRNA group was significantly lower than that in blank control and negative control ($F = 184.27$, $P < 0.05$; $t = 14.87$, $P < 0.01$; $t = 26.53$, $P < 0.01$). Results of

Impact of PKM2 gene silencing on biological behavior of HepG2 cells

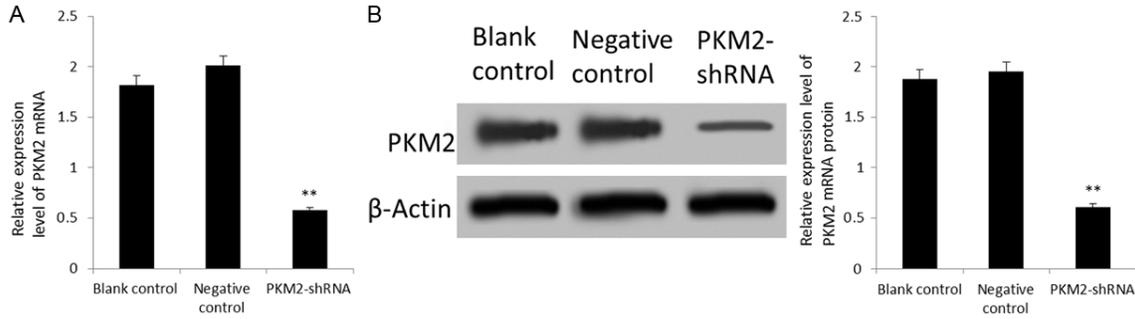


Figure 2. The expression levels of PKM2 mRNA and protein in HepG2 cells transfected with recombinant plasmid pGIPZ-PKM2-shRNA were detected by real-time fluorescent quantitative-PCR (A) and Western blotting (B), respectively. ** $P < 0.01$, vs the blank control and the negative control groups ($n = 3$).

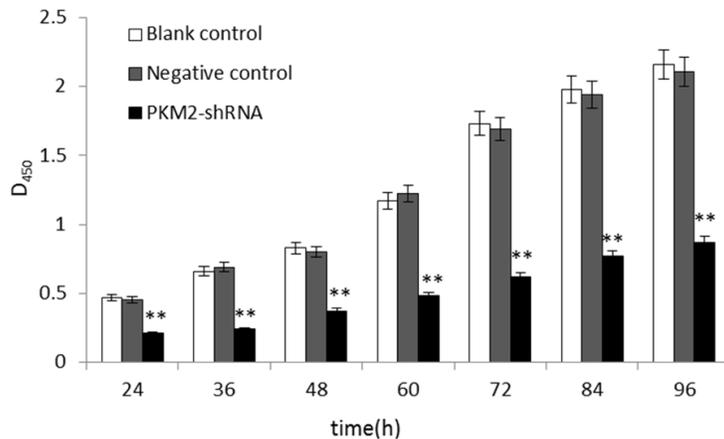


Figure 3. The effects of pyruvate kinase M2 (PKM2)-shRNA on proliferation ability of HepG2 cells were detected by CCK-8 method. ** $P < 0.01$, vs the blank control and the negative control groups ($n = 3$).

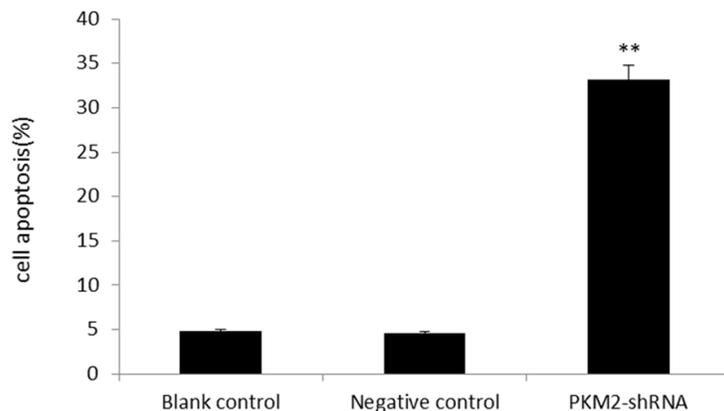


Figure 4. The effects of pyruvate kinase M2 (PKM2)-shRNA on apoptosis ability of HepG2 cells were detected by FCM. ** $P < 0.01$, vs the blank control and the negative control groups ($n = 3$).

Western Blotting (**Figure 2B**) indicated that the expression level of PKM2 proteins in HepG2 cells in PKM2-shRNA group was significantly

lower than that in blank control and negative control and the difference was statistically significant ($F = 21.50$, $P < 0.01$; $t = 4.69$, $P < 0.01$; $t = 5.29$, $P < 0.01$). It showed that PKM2-shRNA transfection could inhibit the expression of PKM2 mRNA and proteins in HepG2 cells effectively.

The proliferation of HepG2 cells could be inhibited by down-regulating PKM2 expression

Results of detection performed by CCK-8 method (**Figure 3**) suggested that D values of HepG2 cells in PKM2 shRNA group detected at seven time points 24, 36, 48, 60, 72, 84 and 96 h were obviously lower than those in blank control and negative control ($F = 6.58$, $P < 0.05$). The proliferation inhibition rates of HepG2 cells at these time points were (28.34±1.23)%, (34.67±2.18)%, (40.88±2.76)%, (45.67±3.41)%, (51.55±2.79)%, (58.38±3.11)% and (64.71±3.82)%, respectively in PKM2-shRNA group; (3.14±0.41)%, (2.76±0.18)%, (2.34±0.34)%, (2.11±0.21)%, (1.89±0.11)%, (1.62±0.15)% and (1.34±0.09)% in negative control; and (2.38±0.43)%, (1.87±0.72)%, (2.17±0.66)%, (1.43±0.52)%, (2.02±

0.19)%, (1.88±0.84)% and (1.65±0.45)% in blank control. The proliferation inhibition rate of HepG2 cells in PKM2 shRNA group was signifi-

Impact of PKM2 gene silencing on biological behavior of HepG2 cells

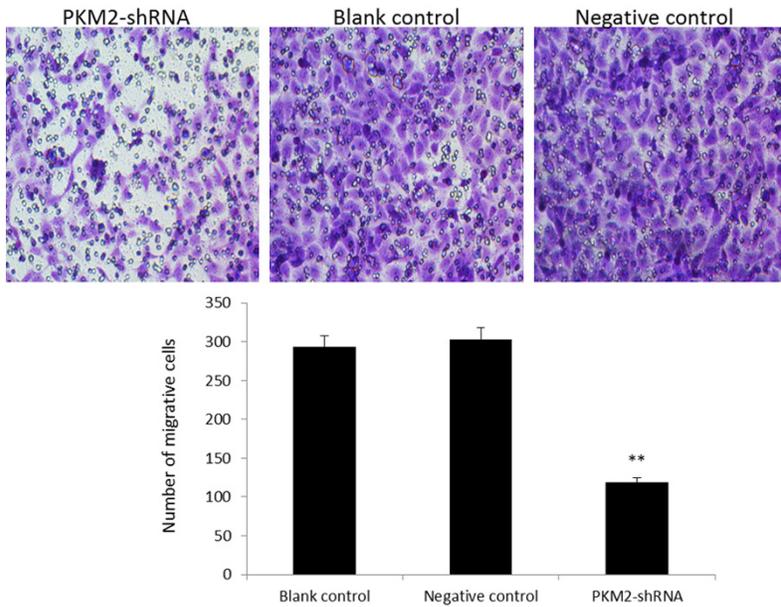


Figure 5. The effects of pyruvate kinase M2 (PKM2)-shRNA on invasion ability of HepG2 cells were detected by Transwell invasion test (crystal violet staining, $\times 200$), respectively. $^{**}P < 0.01$, vs the blank control and the negative control groups ($n = 3$).

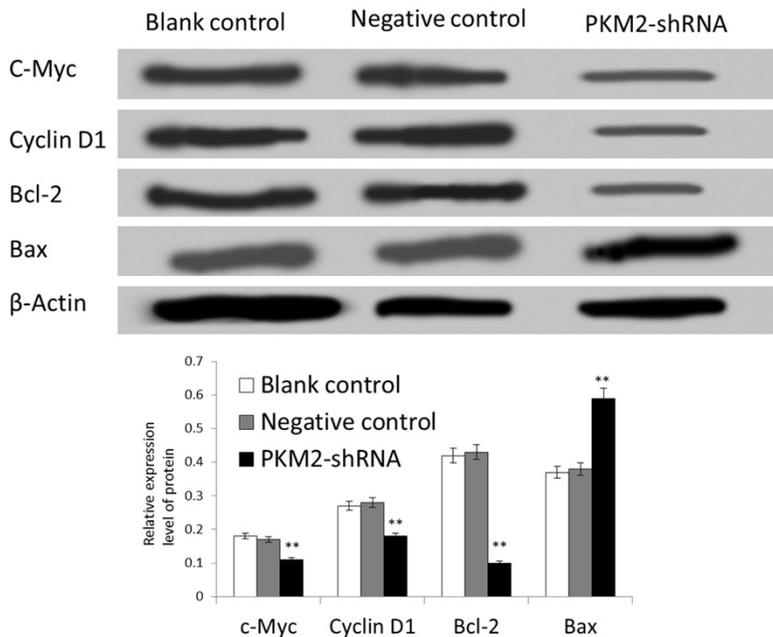


Figure 6. The expression levels of proliferation-related proteins c-myc and cyclin D1 and apoptosis-related proteins Bcl-2 and Bax in HepG2 cells after transfection with PKM2-shRNA were detected by Western blotting. $^{**}P < 0.01$, vs the blank control and the negative control groups ($n = 3$).

cantly higher than that in negative control and blank control. The difference was of statistical significance ($F = 276.5$, $P < 0.05$). It suggested

that the proliferation of HepG2 cells was evidently inhibited after PKM2-shRNA specific interference with PKM2 expression.

The apoptosis of HepG2 cells could be promoted by down-regulating PKM2 expression

Results of detection performed by FCM method (Figure 4) showed that apoptosis rates in blank control, negative control, and PKM2-shRNA group were $(4.82 \pm 0.38)\%$, $(4.59 \pm 0.27)\%$ and $(33.11 \pm 1.87)\%$, respectively. Compared with negative control, the apoptosis rate in PKM2-shRNA group was significantly higher and the difference was of statistical significance ($t = 17.45$, $P < 0.01$). By contrast, the difference between blank control and negative control was of no statistical significance ($t = 0.24$, $P = 0.69$). It indicated that the apoptosis of HepG2 cells was promoted by PKM2-shRNA specific interference with PKM2 expression.

The invasion of HepG2 cells could be inhibited by down-regulating PKM2 expression

Results of Transwell invasion assay (Figure 5) indicated that the average number of cells passing through membrane was (293 ± 5) , (303 ± 4) and (119 ± 3) in blank control, negative control and PKM2-shRNA group, respectively. Compared with blank control and negative control, the number of

cells passing through membrane in PKM2-shRNA group decreased significantly and the difference was of statistical significance ($F =$

Impact of PKM2 gene silencing on biological behavior of HepG2 cells

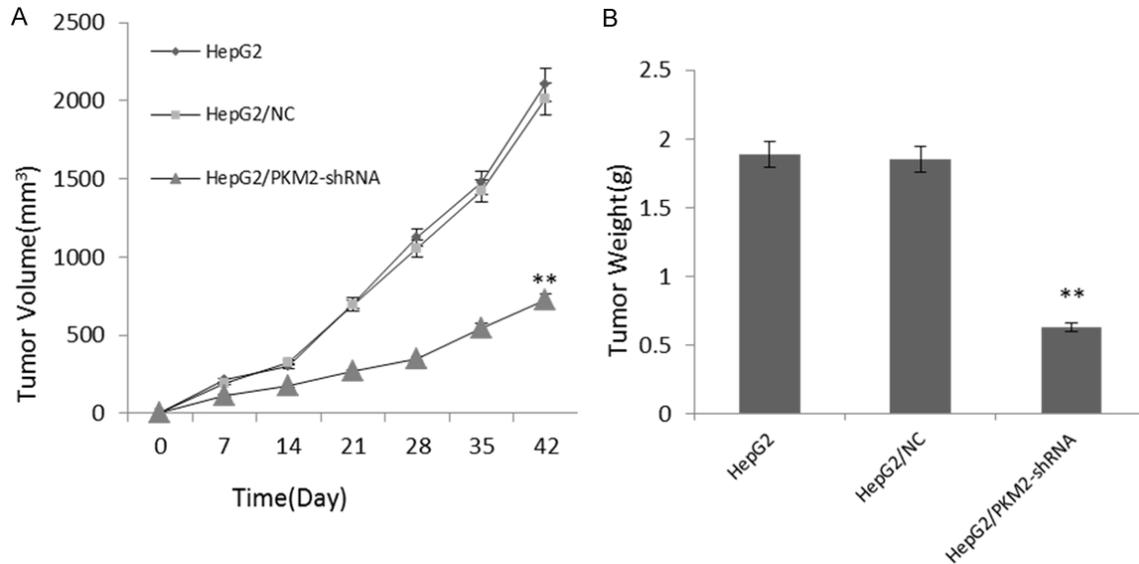


Figure 7. Effects of pyruvate kinase M2 (PKM2)-shRNA on the in vivo tumor growth rate for the HepG2 cells. A: Growth of tumor for the HepG2, HepG2/NC and HepG2/PKM2-shRNA cells after subcutaneous inoculation in BALB/C nude mice. B: At 42 days after inoculation, the mice were sacrificed and dissected, with the weight of tumor measured. Weight of tumor is expressed in mean \pm SD (n = 4), **P < 0.01.

25.23, P < 0.01; t = 43.94, P < 0.01; t = 54.08, P < 0.01). It suggested that the invasion ability of HepG2 cells was inhibited evidently after PKM2-shRNA specific interference with PKM2 expression.

The expression of c-myc, cyclin D1, Bcl-2 and Bax

Results of detection performed by Western Blotting (Figure 6) indicated that, 48 h after transfection with PKM2-shRNA, the expression level of proteins c-myc, cyclin D1 and Bcl-2 in PKM2-shRNA group was obviously lower than that in blank control and negative control and the difference was of statistical significance (P < 0.05). By contrast, the expression level of Bax was evidently higher than that in negative control and the difference was of statistical significance (P < 0.05). It demonstrated that, after PKM2-shRNA specific interference with PKM2 expression, the proliferation could be inhibited by down-regulating c-myc and cyclin D1 expression and the apoptosis could be promoted by down-regulating Bcl-2 expression and up-regulating Bax expression.

Anti-tumor effect in vivo

To investigate the impact of PKM2 over-expression on tumor growth rate in HepG2 cells, we

established a subcutaneous tumor model in BALB/C nude mice and observed tumor formation and growth in HepG2, HepG2/NC and HepG2/PKM2-shRNA cells in this model. Results (Figure 7A) showed that lung cancer lumps were detected subcutaneously 14 days after hypodermic inoculation, whereafter tumor grew slowly. 42 days after inoculation, tumor volume in HepG2/PKM2-shRNA group reached up to (723.3 \pm 125.4) mm³, which was lower than (2103.5 \pm 35.5) mm³ in HepG2 group and (2009.4 \pm 157.9) mm³ in HepG2/NC group. Statistical analysis indicated that there was no statistically significant difference between the tumor growth rate in HepG2 group and that in HepG2/NC group (P > 0.05). By contrast, the tumor growth rate in HepG2/PKM2-shRNA group was significantly lower than that in HepG2 group (P < 0.05) and HepG2/NC group (P < 0.05). After execution, their tumors were weighed and results showed that tumor mass in HepG2/PKM2-shRNA group was (0.63 \pm 0.14) g, which was significantly lower than (1.89 \pm 0.23, P < 0.05) in HepG2 group and (1.85 \pm 0.12, P < 0.05) in HepG2/NC group (Figure 7B).

Discussion

In recent years, as researches and understanding about cancer went deeper, tumor metabolism became the focus of attention. Unlike nor-

Impact of PKM2 gene silencing on biological behavior of HepG2 cells

mal cells, tumor cells mainly supplied energy by glycolysis instead of aerobic oxidation. In other words, "Warburg effect" increased in tumor cells [13]. Hence, a great number of metabolites and metabolic enzymes increased in tumor cells, including lactic acid, pyruvate kinase and Monoacylglycerol lipase [13-15]. The role of these abnormally increased substances in cancer progression was gaining attention from more and more researchers. PKM2 was a subtype of PK. It was reported that abnormally increased PKM2, which was a key enzyme of metabolism, could enhance "Warburg effect" in tumor cells [16]. In addition, studies found that PKM2 also could be transfected into nucleus as a transcriptional coactivator and impact the transcription of downstream genes after combining with other transcriptional coactivators, such as SP1, peroxisome proliferator-activated receptor- γ (PPAR γ), hypoxia-inducible factor-1 α (HIF-1 α), nuclear factor-kappa B (NF- κ B) and OCT4 [17, 18]. However, there have been few studies on the role of PKM2 in HCC. Therefore, by taking PKM2 as a research target, this study investigated the impact of down-regulating PKM2 expression on the proliferation, invasion and apoptosis of HepG2 cells by shRNA specific interference with PKM2 expression.

In this study, first of all, the high expression of PKM2 in HepG2 cells was demonstrated by immunofluorescence. It provided a basis for selecting this cell line as our study object in the following step. Then, HepG2 cells were transfected with PKM2-shRNA recombinant plasmid by liposome method. 48 h later, the expression level of PKM2 mRNA and proteins were detected. Real-time fluorescence quantification PCR and Western Blotting showed that PKM2-shRNA inhibited the expression of PKM2 mRNA and proteins in HepG2 cells effectively. In the following part, this study discussed the impact of down-regulating PKM2 expression on the proliferation, apoptosis and invasion of HepG2 cells. Results of detection by CCK-8 method and Transwell invasion assay indicated that silencing PKM2 gene expression specifically by PKM2-shRNA transfection inhibited the proliferation and invasion of HepG2 cells effectively. Furthermore, results of detection by FCM method showed that silencing PKM2 gene could also promote the apoptosis of HepG2 cells. They were consistent with findings of studies on PKM2 in other cancers like gastric cancer, glioma

and thyroid cancer [19-21]. To further explore the mechanism of how PKM2 impacted the proliferation and apoptosis of HepG2 cells, we analyzed the expression level of proliferation-associated proteins c-myc and cyclin D1 and apoptosis-associated proteins Bcl-2 and Bax 48 h after PKM2-shRNA transfection by Western Blotting. It was found that PKM2 gene silencing up-regulated the expression of c-myc, cyclin D1 and Bcl-2 and down-regulated the expression of Bax. In conclusion, PKM2-shRNA could inhibit the proliferation and invasion of HepG2 cells and promote their apoptosis by down-regulating the expression of PKM2. However, further studies were yet to be conducted to find out the signal pathway by which the expression of downstream target genes c-myc, cyclin D1, Bcl-2 and Bax was regulated.

By taking the impact of PKM2 on the proliferation, apoptosis and metastasis of hepatoma cells as a starting point, this study found that interference with PKM2 expression could inhibit the proliferation and metastasis of HCC cells and promote their apoptosis. It provided a new target and thought for HCC treatment. Since PKM2 was both a key enzyme and important transcriptional coactivator during metabolism, whether HCC could be treated by target interference with PKM2 expression needs further investigation. Moreover, it was reported that after interference with PKM2 expression, signal pathway phosphoinositide-3 kinase (PI3K)-protein kinase B (PKB, also known as Akt) was activated [22]. Hence, further studies also need to be conducted to confirm whether HCC could not be treated by simple interference with PKM2 expression.

Disclosure of conflict of interest

None.

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Impact of PKM2 gene silencing on biological behavior of HepG2 cells

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