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Overexpression of Pyruvate Kinase Type M2 (PKM2) Promotes Ovarian Cancer Cell Growth and Survival Via Regulation of Cell Cycle Progression Related with Upregulated CCND1 and Downregulated CDKN1A Expression

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: Many findings have shown that pyruvate kinase type M2 (PKM2) plays crucial roles in regulating the occurrence and development of various human cancers; however, its roles in ovarian cancer oncogenesis remain to be determined.

Material/Methods: The expression intensity of PKM2 in ovarian cancer tissues was examined by immunohistochemistry (IHC), and was then correlated to patient clinicopathologic characteristics. The roles of PKM2 in ovarian cancer cell proliferation, growth, and survival were examined by CCK-8, colony forming, and flow cytometry assays. The potentially involved molecular were then investigated by Western blot analysis.

Results: IHC results showed that PKM2 was overexpressed in 100 of 114 (87.7%) serous ovarian cancer tissues as compared with 50 cases of non-cancerous ovarian tissues, and was associated with tumor size ≥7.5 cm and <7.5 cm ($p<0.05$). Overexpression of PKM2 in SKOV3 and HEY ovarian cancer cells by transfection with PKM2 lentivirus vector led to increased cell proliferation, growth, and survival, which may be related with PKM2 being able to increase cell cycle progress: G1 stage decreased, whereas S stage significantly increased. In contrast, all functions of SKOV3 and HEY cells described above were reversed by knocked down PKM2 expression using siRNA. Further data showed that overexpressed PKM2 led to increased CCND1 and decreased CDKN1A expression, whereas underexpressed PKM2 led to decreased CCND1 and increased CDKN1A expression in ovarian cancer cells.

Conclusions: PKM2 may play important roles in ovarian cancer development and may be a treatment target for this cancer.

MeSH Keywords: Cell Proliferation • Cell Survival • Cyclin D1 • Cyclin-Dependent Kinase Inhibitor p21 • Ovarian Neoplasms • Pyruvate Kinase

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Background

Ovarian cancer is one of the most common gynecologic cancers, and its incidence is third highest among the gynecologic malignant tumors. Patient survival is usually poor, and the 5-year survival rate is still poorer [1]. To date, the molecular mechanisms involved in ovarian cancer incidence and development remain largely unknown. While numerous studies have shown that aberrant oncogene expression and their products or key signal molecules, including protein kinases, may play essential roles in cancer development [2], the exact pathways involved in ovarian cancer remain to be determined. Investigation of the molecular mechanisms of ovarian cancer occurrence, development, invasion, metastasis, and survival will be of great value for providing a foundation for clinical treatment and for studying the prognosis of patients with ovarian cancer. In recent years, an increasing number of findings have shown that abnormal expression of pyruvate kinase type M2 (PKM2) is closely associated with tumor progression [3–7], but whether PKM2 is related to ovarian cancer remains unclearly. Therefore, in this study, we investigated the expression of PKM2 in ovarian cancer tissues, and the effect of PKM2 on ovarian cancer cell proliferation, growth, and survival, as well as the potential mechanism related with PKM2 on ovarian cancer development, by overexpressing and underexpressing PKM2. Our results may form the basis for further understanding the molecular mechanisms of ovarian cancer incidence and development.

Material and Methods

Tissue samples

We obtained 114 paraffin-embedded serous ovarian cancer tissue samples and 50 paraffin-embedded non-cancerous ovarian tissue samples from patients who underwent surgical resection between January 2003 and December 2010 at Fuzhou General Hospital and the Cancer Hospital of Fujian Province (Fujian, China). All samples had been diagnosed pathologically and classified by doctors at the Departments of Pathology of both hospitals.

Ethics statement

The Ethics Committees of Fuzhou General Hospital and the Cancer Hospital of Fujian Province approved this study. In addition, all patients consented to the use of their tissue samples for research.

Immunohistochemistry (IHC) for PKM2

Anti-PKM2 antibody was purchased from Abcam (Cambridge, MA, USA). The other IHC reagents were EliVision plus immunohistochemical staining reagent, citric acid antigen repair solution, concentrated diaminobenzidine (DAB) color reagent kit, and hematoxylin restaining reagent, which were all purchased from Maixin (Fujian, China). IHC detection of PKM2 expression in the cancer and non-cancerous tissues was performed based on the method by Liu et al. for human EGFR 2 (HER2) in gastric cancer [8]. All results were evaluated by pathologists using a semi-quantitative method. Based on the degree of tissue section staining, the results were divided into 4 grades: –, no staining; +, weak staining; ++, moderate staining; and +++, strong staining. Staining intensity ≥ + was considered positive. PKM2 expression was determined by ratios, where PKM2 expression levels in the cancer tissue were divided by that in non-cancerous tissues. A ratio >1 indicated PKM2 overexpression in the cancer tissues; a ratio =1 indicated no significant difference between PKM2 expression levels in the cancer and non-cancerous tissues; and a ratio <1 indicated downregulated PKM2 in the cancer tissues.

PLVX-Neo-IRES-ZsGreen1-PKM2 expression vector construct

The pLVX-Neo-IRES-ZsGreen1 lentivirus vector was purchased from Genomeditech (Shanghai, China), and the pDsRed-Express-C1-PKM2 plasmid (a gift from Professor RN Bamezai) was used as the template. To clone human *PKM2* cDNA into the pLVX-Neo-IRES-ZsGreen1 vector, *Xho*I and *Not*I restriction sites were inserted into the PCR products using the following primers: 5'-CCGCTCGAGATGTCGAAGCCCCATAGTGAAG-3' (forward) and 5'-ATAAGAATGCGGCCGCTCACGGCACAGGAACAACACGC-3' (reverse). After confirming the PKM2 sequences by sequencing, the plasmid was cotransfected into 293T cells with the lentivirus packaging plasmids pMD2.G and psPAX2 to produce lentivirus particles. The PKM2 lentivirus particles were then used to transduce SKOV3 and HEY ovarian cells. SKOV3 and HEY cells stably overexpressing PKM2 or empty vector were established by first transducing the cells with PKM2 lentivirus particles or empty vector lentivirus particles, and then screening them using G418.

Silencing PKM2 expression in SKOV3 and HEY cells with PKM2 siRNA

SKOV3 and HEY cells which exhibited low-expressed PKM2 were obtained through silencing PKM2 by transfected with PKM2 siRNA (targeting on the sites of 1280–1298 bp of PKM2) (sense: CCAUAAUCGUCCUCACCAATT, antisense: UUGGUGAGGACGAUUAUGGTT, purchased from RUIBIO Co., Guangzhou, China). At the same time, scramble siRNA (negative siRNA) (Sense: AAACCUUUCCCCCUAAACGTT, antisense: CGUUUAGGGGGAAAGGUUUTT), which was also obtained from the same company, was transfected into SKOV3 and HEY cells, respectively, to serve as negative controls.

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Western blot detection of PKM2, CCND1, and CDKN1A expression in SKOV3 and HEY cells

PKM2, CCND1, and CDKN1A expression in SKOV3 and HEY cells were detected by Western blotting, which was based on our previously described methods [9], but the ECL substrate used was Pierce™ ECL Western Blotting Substrate and the results were detected by using ChemiDoc™ Touch Imaging System (Bio-Rad Company, USA). The anti-PKM2 antibody (1: 1000) used for the Western blotting was the same as that used for IHC. The anti-CCND1 (1: 1000) and anti-CDKN1A (1: 1000) used for the Western blotting were purchased from ZSGB-BIO (Beijing, China) and Santa Cruz (CA, USA), respectively. β-Actin antibody (1: 4000) serving as the control was purchased from abMart (Shanghai, China).

CCK-8 assay detection of SKOV3 and HEY cell proliferation

SKOV3 and HEY cells were divided into 5 groups: untransfected cells, transduced with empty vector lentivirus particles, transduced with PKM2 lentivirus particles, transfected with scramble siRNA (negative siRNA), and transfected with PKM2 siRNA. The cells were seeded in 96 well-plates (cells, 4000 cells/well; culture medium, 100 µL/well). Seeding per group was repeated in 6 different wells. After overnight culture in a 37°C incubator with 5% CO_2, the cells were grown in culture medium containing 2% fetal bovine serum (FBS) for a total 72 h; during this period, CCK-8 assays were performed at 6, 24, 48, and 72 h, respectively, which were achieved by adding CCK-8 (10 µL, 5 mg/mL) (Genview, Beijing, China) to each well, and cells were cultured for another 1 h. The blank contained culture medium and CCK-8. The absorbance at 450 nm (A450) against that of the blank was measured using a microplate reader (Bio-Tek, VT, USA).

Colony forming assay to detect SKOV3 and HEY cell growth

A density with 500 SKOV3 and HEY cells/per well of a 6-well plate was applied to grow SKOV3 and HEY cells, respectively, to perform colony forming assays. After cells had grown for 14 days, the formed cell clones were checked. The clones were then fixed by menthol and stained with 0.1% crystal violet at room temperature. Clones containing 50 or more than 50 cells were regarded as a positive clone when observed under a microscope, and the colony forming rate=numbers of positive clones/total cell seeded in the well×100%.

Annexin V-FITC+Propidium Iodide staining combined flow cytometry assay to detect SKOV3 and HEY cell apoptosis

Annexin V-FITC+Propidium Iodide (PI) kits for detection of cell apoptosis were purchased from KeyGen Biotech Company (Nanjing, Jiangsu, China). Cell apoptosis in SKOV3 and HEY cells were examined with flow cytometry by staining with the kit according to the manufacturer's instructions.

PI staining combined flow cytometry assay to detect SKOV3 and HEY cell cycle

Propidium Iodide (PI) staining kits for cell cycle were purchased from Beyotime Institute of Biotechnology (Haimen, Jiangsu, China). Cell cycle was analyzed by flow cytometry after SKOV3 and HEY cells had been stained with the kit according to the manufacturer's instruction.

Statistical analysis

Statistical differences in PKM2 expression levels between the serous ovarian cancer tissues and non-cancerous tissues were determined by the Wilcoxon signed ranks test. The chi-square test and Fisher's exact test were used when necessary to analyze the clinical significance of differences between PKM2 expression levels and patient clinicopathological features. The single-factor analysis of variance (ANOVA) and independent *t* test were used to analyze differences in the proliferation, colony forming, apoptosis, and different stages of cell cycle in the different groups of SKOV3 and HEY cells. All analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). $p<0.05$ was considered statistically significant.

Results

PKM2 was overexpressed in serous ovarian cancer tissues

The IHC results showed that among the 114 serous ovarian cancer tissues, 100 (87.7%) had positive PKM2 expression. By contrast, only 4 of the 50 non-cancerous ovarian tissues had positive PKM2 expression. The PKM2 expression levels between the cancer and non-cancerous tissues were statistically significantly different (P<0.001) (Figure 1). Subsequently, PKM2 expression in the cancer tissues was divided into low expression (PKM2 expression of – or +) and high expression (PKM2 expression of ++ or +++) groups. The results showed that PKM2 expression was not related with patient age, sex, tumor-node-metastasis (TNM) stage, or metastasis (all, $p>0.05$), but was related with tumor size (≥7.5 cm *vs.* <7.5 cm, P=0.046), where more patients with high PKM2 expression had tumors ≥7.5 cm (25/61, 40.98% *vs.* 11/48, 22.92%, P=0.046) among 109 cases of patients who had the available data of tumor size. These results suggest that PKM2 overexpression may be related to ovarian cancer development and growth.

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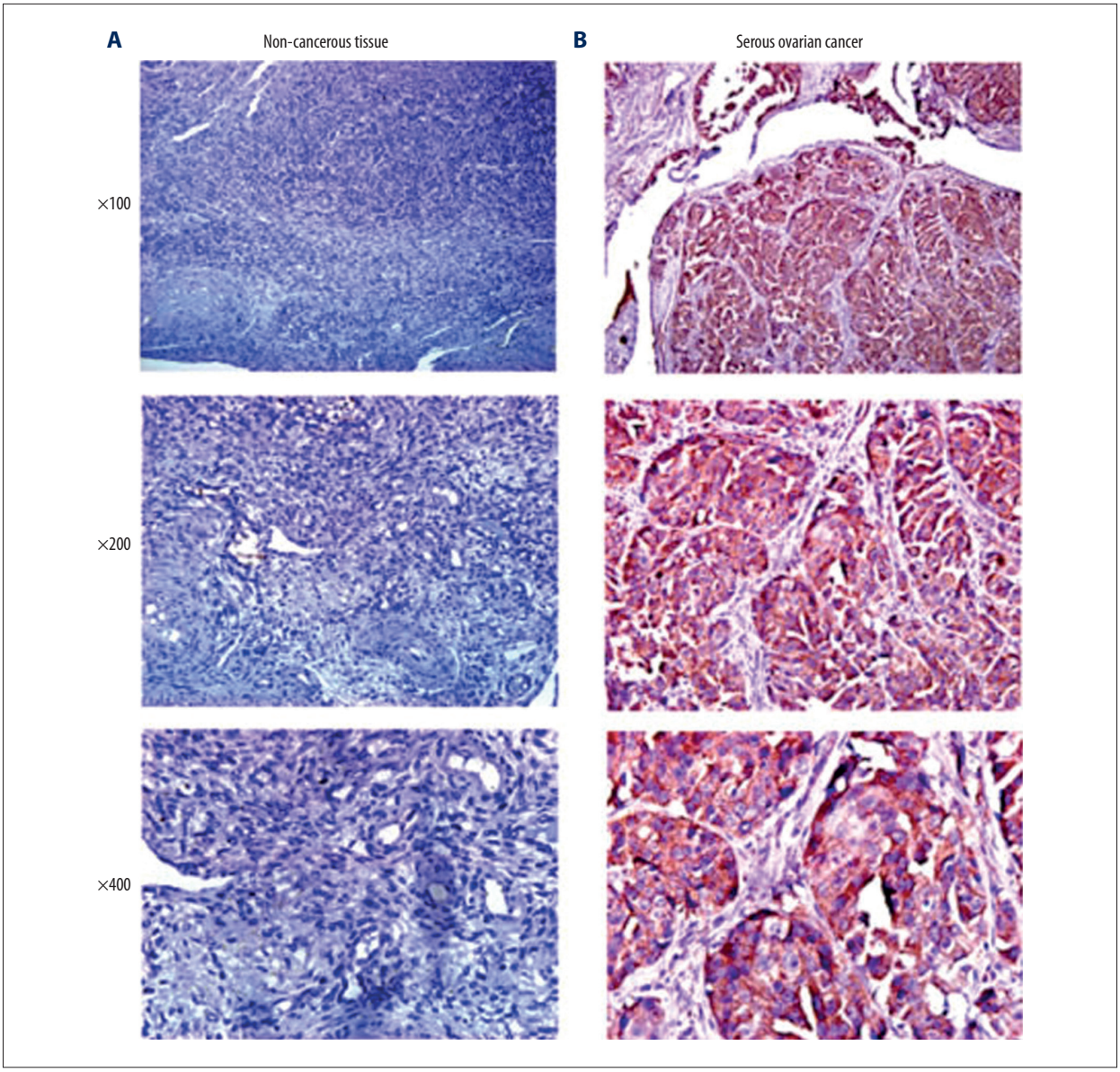

Figure 1. IHC detection of PKM2 overexpression in serous ovarian cancer tissues compared to that in non-cancerous tissues. (**A**) PKM2 expression in non-cancerous tissue. (**B**) PKM2 expression in serous ovarian cancer tissue.

PKM2 overexpression increased SKOV3 and HEY cell proliferation

The CCK-8 assay showed that PKM2 overexpression significantly increased SKOV3 cell and HEY cell proliferation, with the highest increased peak at 72 h at the determined time periods in this study, compared to those of empty vector transduction or wild-type cells ($p<0.05$) after cells had grown for 48 h, whereas no statistically significant difference was detected between empty vector transduction cells and wild-type cells in terms of cell proliferation (Figure 2A, 2B). Silenced PKM2 expression by siRNA significantly inhibited SKOV3 and HEY cell proliferation compared to those in both SKOV3 and HEY cells transfected with scramble siRNA (negative siRNA), both $p<0.05$, after cells had grown for 48 h (Figure 2A, 2B). The results indicate that PKM2 overexpression can lead to increased ovarian cancer cell proliferation, whereas PKM2 downexpression can lead to decreased ovarian cancer cell proliferation.

PKM2 overexpression increased SKOV3 and HEY cell growth

Colony forming assay showed that PKM2 overexpression significantly increased SKOV3 cell and HEY cell growth compared to those of empty vector transduction, both p<0.05 (Figure 3A–3D). Similar to results of cell proliferation assays, no statistically significant difference was detected between empty vector

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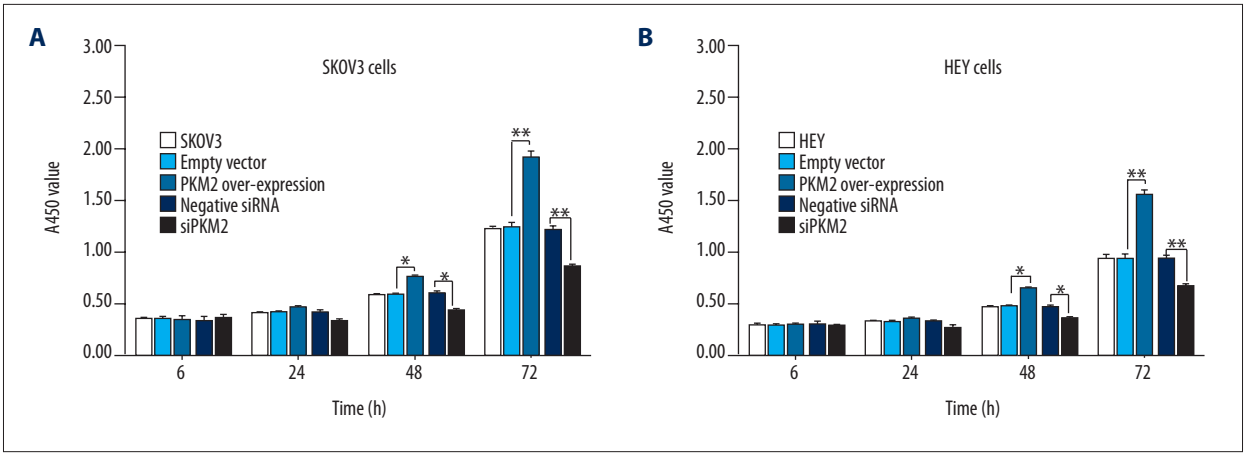

Figure 2. CCK-8 assay detection of SKOV3 and HEY cell proliferation. PKM2 lentivirus expression vector transduction increased SKOV3 and HEY cell proliferation compared to that of empty vector transduction or wild-type cell ($p<0.05$); proliferation was not changed in empty vector transduced SKOV3 and HEY cells as compared to untransfected SKOV3 cells or HEY cells (P>0.05). PKM2 siRNA transfection decreased SKOV3 and HEY cell proliferation compared to that of scramble siRNA (negative siRNA) transfection ($p<0.05$). (**A**) SKOV3 cell proliferation results; (**B**) HEY cell proliferation results. * $p<0.05$; ** $p<0.01$.

transduction cells and wild-type cells in cell growth detected by colony forming assays (Figure 3A–3D). Silenced PKM2 expression by siRNA significantly inhibited SKOV3 and HEY cell growth compared to those in both SKOV3 and HEY cells transfected with scramble siRNA (negative siRNA), both $p<0.05$ (Figure 3A–3D). The results indicate that PKM2 overexpression can lead to increased ovarian cancer cell growth, whereas PKM2 downexpression can lead to decreased ovarian cancer cell growth.

PKM2 overexpression decreased SKOV3 and HEY cell apoptosis

Annexin V-FITC+Propidium Iodide staining combined flow cytometry assay showed that PKM2 overexpression decreased SKOV3 cell and HEY cell apoptosis compared to those in empty vector transduction and wild-type cells, both $p<0.05$ (Figure 4A–4D), whereas no statistical significant difference was detected between empty vector transduction cells and wild-type cells in cell apoptosis (Figure 4A–4D). Silenced PKM2 expression by siRNA significantly increased SKOV3 and HEY cell apoptosis compared to those in both SKOV3 and HEY cells transfected with scramble siRNA (negative siRNA), both $p<0.05$ (Figure 4A–4D). The results indicate that PKM2 overexpression can lead to decreased ovarian cancer cell apoptosis, whereas PKM2 downexpression can lead to increased ovarian cancer cell apoptosis.

PKM2 overexpression increased ovarian cancer cell proliferation, growth, and survival via increased S stage of cell cycle progression

Propidium iodide staining combined flow cytometry assay cell cycle showed PKM2 overexpression significantly increased S stage of cell cycle progression in SKOV3 cells and HEY cells, compared to those in empty vector transduction and wild-type, both $p<0.05$ (Figure 5A–5D), whereas no statistically significant difference was detected between empty vector transduction cells and wild-type cells in S stage of cell cycle progression (Figure 5A–5D). Silenced PKM2 expression by siRNA significantly decreased S stage of cell cycle progression in SKOV3 and HEY compared to those in SKOV3 and HEY cells transfected with scramble siRNA (negative siRNA), both $p<0.05$ (Figure 5A–5D). Additionally, overexpression of PKM2 also decreased G1 stage, and low expression of PKM2 also increased G1 stage of cell cycle progression of SKOV3 and HEY cells (Figure 5A–5D). The results indicate that PKM2 overexpression can lead to increased ovarian cancer cell development through regulating cell cycle progression.

PKM2 overexpression increased CCND1 and decreased CDKN1A expression in SKOV3 and HEY cells

The roles of CCND1 and CDKN1A in mediating cell cycle progression have been widely documented [10,11]. Many studies have confirmed that CCND1 mainly has an oncogenic effect, whereas CDKN1A mainly acts as a suppressor of cancer, and both of them are closely linked to development of various human cancers [12,13]. However, the role of PKM2 in promoting ovarian cancer cell cycle progression remains to be determined. As shown in Figure 6A and 6B, Western blotting results showed that CCND1 was upregulated and downregulated in PKM2 overexpressed and underexpressed SKOV3 and HEY cells, respectively; but CDKN1A was downregulated and upregulated in PKM2 overexpressed and underexpressed SKOV3 and HEY cells, respectively. The results indicate that PKM2 overexpression led to increase ovarian cancer cell development via regulating cell cycle progression, and may be associated with its regulation of CCND1 and CDKN1A expression.

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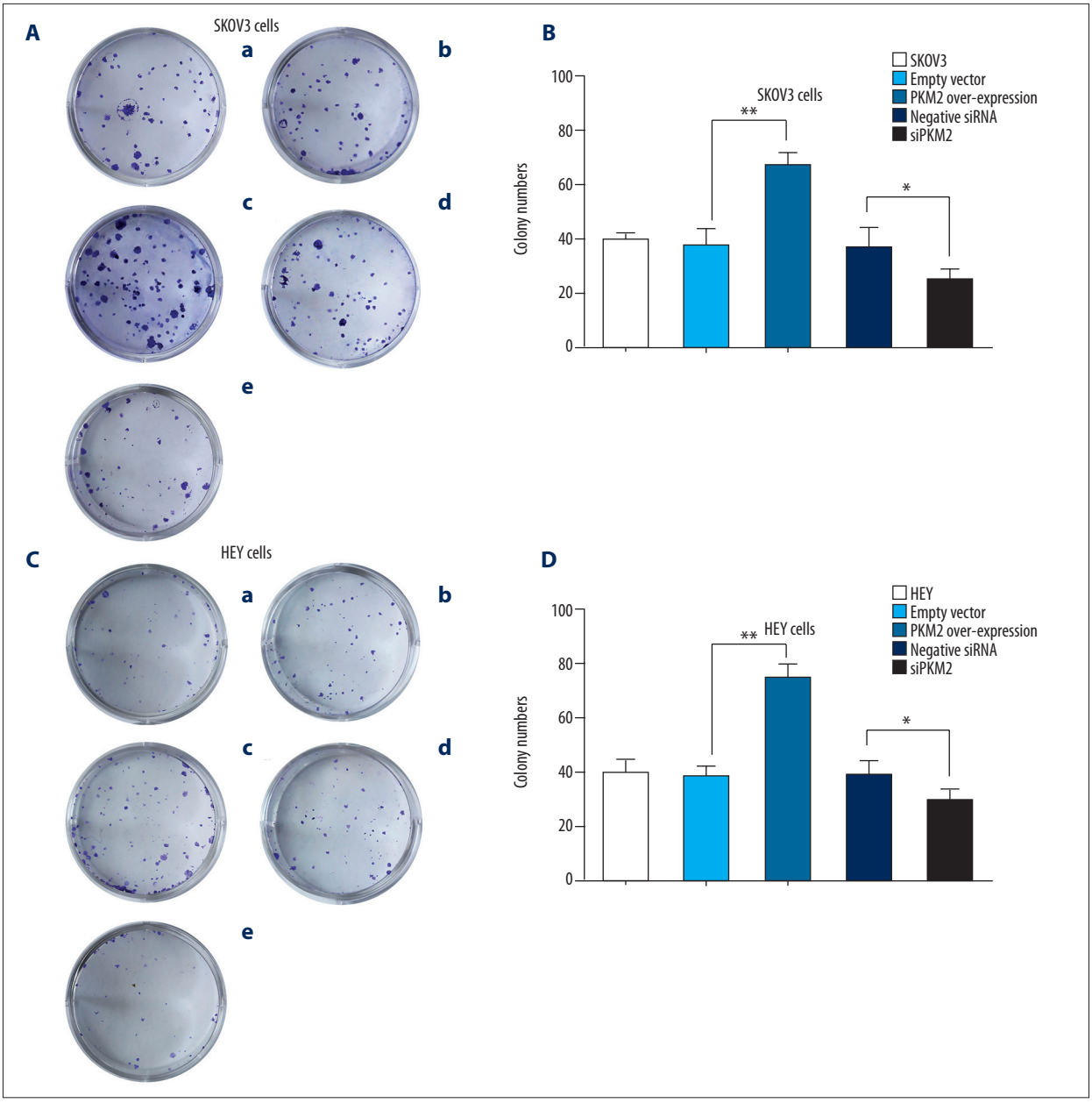

Figure 3. Colony formation assay detection of SKOV3 and HEY cell growth. PKM2 lentivirus expression vector transduction increased SKOV3 and HEY cell growth compared to that of empty vector transduction or wild-type cell ($p<0.05$); Growth was not changed in empty vector transduced SKOV3 and HEY cells as compared to untransfected SKOV3 cells or HEY cells (P>0.05). PKM2 siRNA transfection decreased SKOV3 and HEY cell growth compared to that of scramble siRNA (negative siRNA) transfection ($p<0.05$). (**A, B**) SKOV3 cell colony formation results; (**C, D**) HEY cell colony formation results. * $p<0.05$; ** $p<0.01$. (**a**) Untransfected cells (wild-type cells); (**b**) transduced with empty vector lentivirus particles; (**c**) transduced with PKM2 lentivirus particles; (**d**) transfected with negative siRNA; (**e**) transfected with PKM2 siRNA.

Discussion

PKM2 is a well-known key enzyme of aerobic glycolysis, with high affinity binding with its substrate phosphoenolpyruvic acid (PEP). PKM2 has strong catalytic ability and can catalyze PEP conversion to pyruvate, which is a rate-limiting step of glycolysis, through which it provides energy for cell growth and proliferation. Mammalian cells have 4 pyruvate kinase isozymes – PKM1, PKM2, PKL, and PKR – which are distributed in different tissues and cells. However, in tumor formation, PKM2 gradually replaces the other isozymes to become the major isozyme, and is highly expressed in malignant cells and tissues [10]. PKM2 expression

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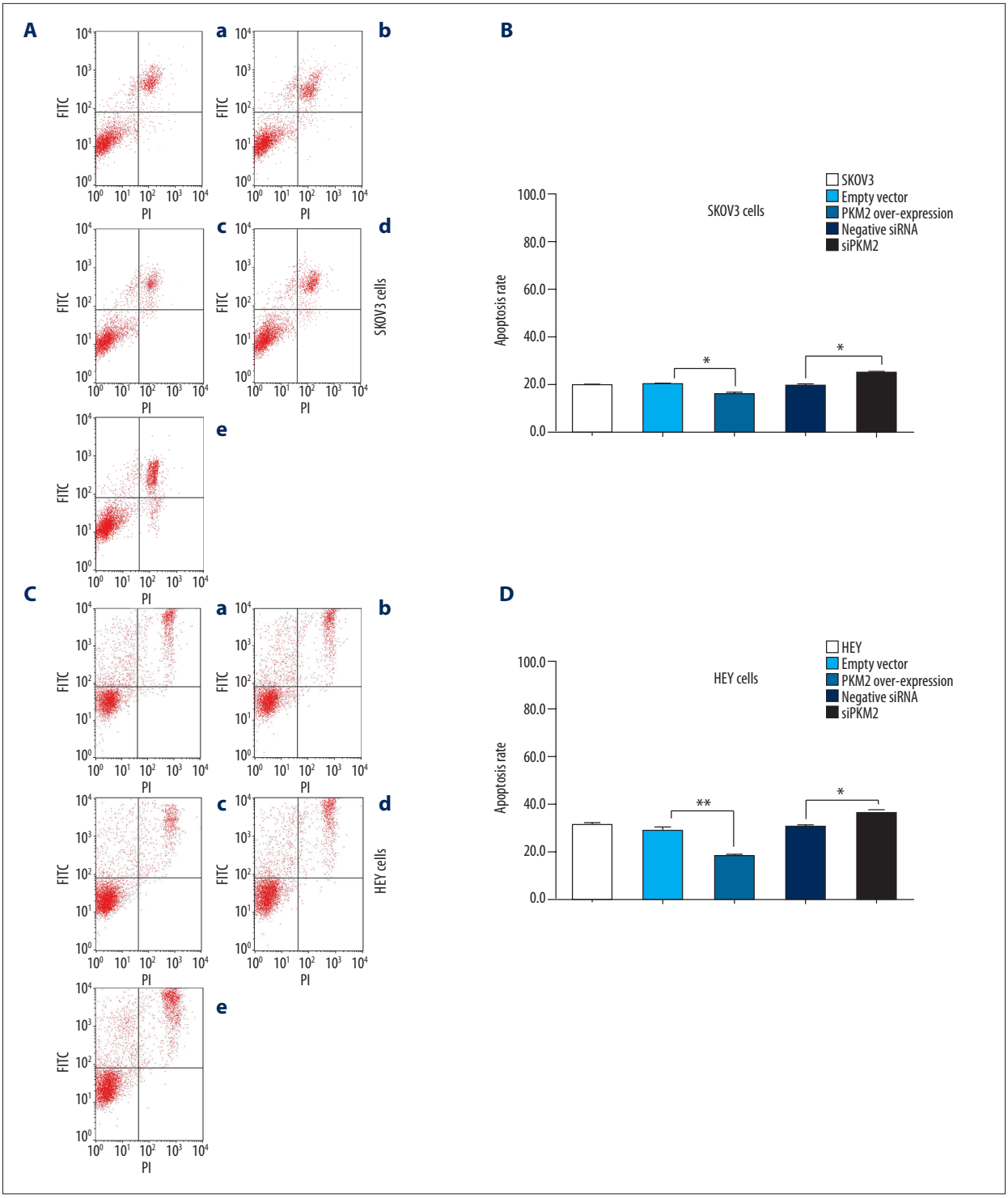

Figure 4. Flow cytometry assay detection of SKOV3 and HEY cell apoptosis. PKM2 lentivirus expression vector transduction decreased SKOV3 and HEY cell apoptosis compared to that of empty vector transduction or wild-type cell ($p<0.05$); Cell apoptosis was not changed in empty vector transduced SKOV3 and HEY cells as compared to untransfected SKOV3 cells or HEY cells ($P>0.05$). PKM2 siRNA transfection increased SKOV3 and HEY cell apoptosis compared to that of scramble siRNA (negative siRNA) transfection ($p<0.05$). (**A, B**) SKOV3 cell apoptosis results; (**C, D**) HEY cell apoptosis results. * $p<0.05$; ** $p<0.01$. (**a**) untransfected cells (wild-type cells); (**b**) transduced with empty vector lentivirus particles; (**c**) transduced with PKM2 lentivirus particles; (**d**) transfected with negative siRNA; (**e**) transfected with PKM2 siRNA.

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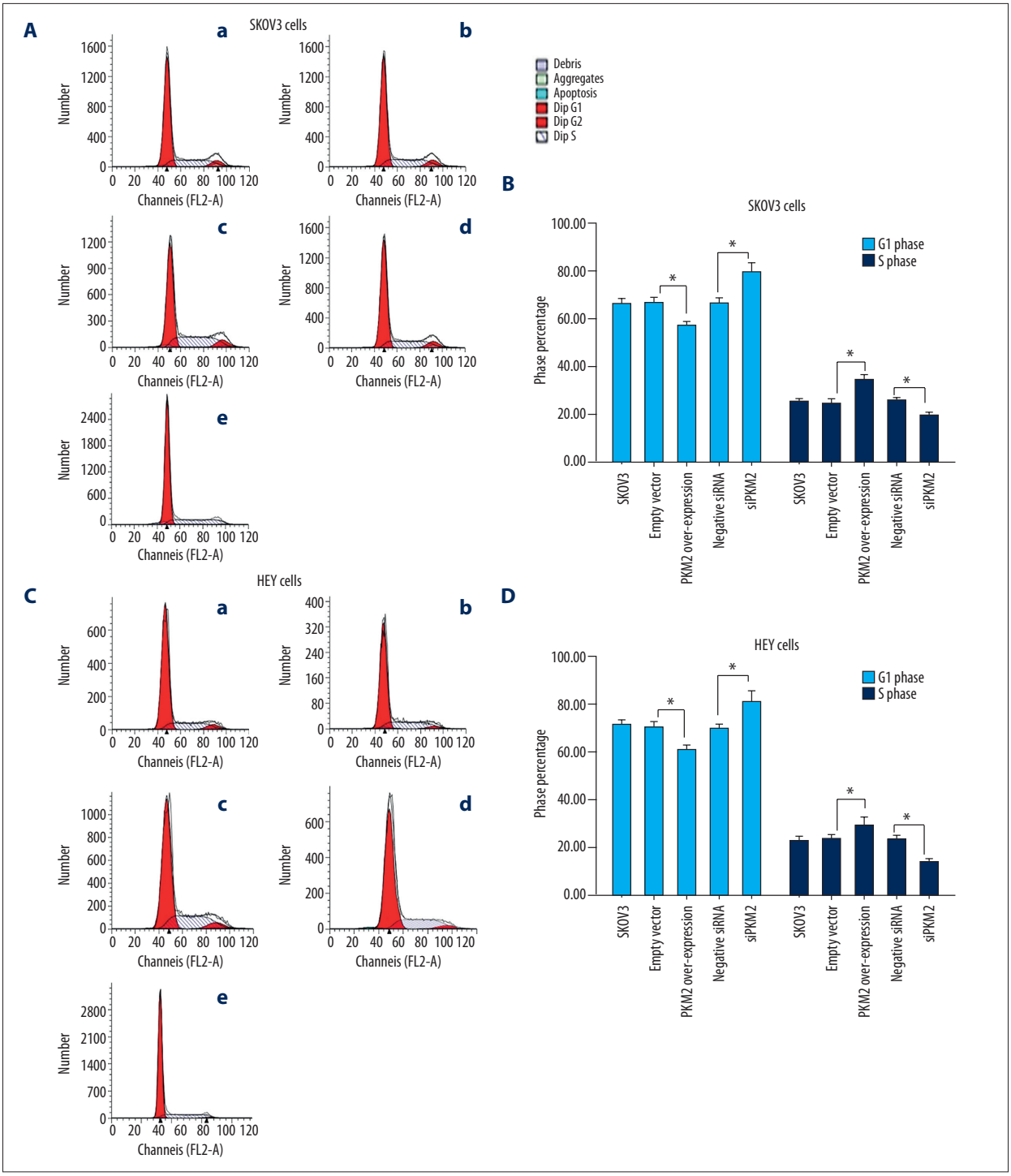

Figure 5. Flow cytometry assay detection of SKOV3 and HEY cell cycles. PKM2 lentivirus expression vector transduction increased the S stage and decreased the G1 stage of the cell cycle in SKOV3 and HEY cell compared to that of empty vector transduction or wild-type cell ($p<0.05$); The stages of cell cycle were not changed in empty vector transduced SKOV3 and HEY cells as compared to untransfected SKOV3 cells or HEY cells (P>0.05). PKM2 siRNA transfection decreased S stage and increased G1 stage in SKOV3 and HEY cell compared to that of scramble siRNA (negative siRNA) transfection ($p<0.05$). (**A, B**) SKOV3 cell cycle results; (**C, D**) HEY cell cycle results. * $p<0.05$; ** $p<0.01$. (**a**) Untransfected cells (wild-type cells); (**b**) transduced with empty vector lentivirus particles; (**c**) transduced with PKM2 lentivirus particles; (**d**) transfected with negative siRNA; (**e**) transfected with PKM2 siRNA.

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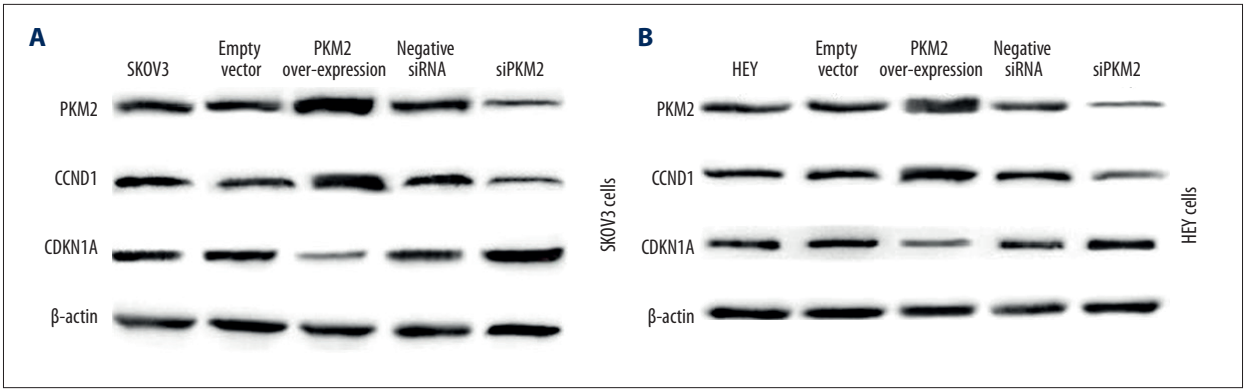

Figure 6. Western blotting assay detection of CCND1 and CDKN1A expression in SKOV3 and HEY cells. PKM2 lentivirus expression vector transduction increased the expression of CCND1 and decreased the expression of CDKN1A in SKOV3 and HEY cells. The expression of CCND1 and CDKN1A was not changed in empty vector transduced SKOV3 and HEY cells as compared to untransfected SKOV3 cells or HEY cells. PKM2 siRNA transfection decreased CCND1 and increased CDKN1A expression in SKOV3 and HEY cell. (**A**) SKOV3 cell results; (**B**) HEY cell results.

is often accompanied by high levels of nucleic acid synthesis, which is frequently observed in almost all proliferating cells (e.g., embryonic cells, adult stem cells, and cancer cells) [14].

Early studies have also consistently demonstrated that PKM2 (the dimeric form of PKM2, also termed TuM2-PK) is a tumor marker whose levels in serum have great value in colon cancer, renal cell carcinoma, and lung cancer diagnosis, therapeutic effect evaluation, treatment monitoring, and prognosis evaluation [15–17]. Recent data from large studies have demonstrated that PKM2 expression is abnormal in many cancer cells and tissues and is closely related to the malignant biological behavior of these cells, and it plays an important role in regulating cancer metabolism and promoting cancer cell growth, proliferation, invasion, and metastasis via various molecular mechanisms [14,18,19]. Zhou et al. showed that PKM2 is overexpressed in colon cancer and is related to both cancer stage and lymph metastasis. Inhibiting PKM2 expression suppressed colon cancer cell proliferation and migration [20]. Kwon et al. indicated that PKM2 is not only overexpressed in gastric cancer tissues, but is also related with shorter survival in gastric cancer patients, which may be related to the capability of PKM2 to increase cell survival by mediating Bcl-xL transcription levels [21]. Liang et al. demonstrated that Cdc25A, a dual-specificity phosphatase, regulates PKM2 dephosphorylation, which in turn increases the Warburg effect, and brain carcinogenesis [22]. Data from the study by Park et al. demonstrated that PKM2 regulation of glycolysis is related to sirtuin type 2 (SIRT2)-associated deacetylation and tetramerization [23]. Park et al. found that the direct interaction of AKT and PKM2 results in Ser202 phosphorylation in PKM2, which then causes PKM2 translocation into the nucleus, where it binds signal transducers and activators of transcription 5A (STAT5A), activating it and leading to increased expression of the oncogene cyclin D1 in response to insulin-like growth factor 1 (IGF1) treatment [24].

Regarding the relationship between EGFR and PKM2, not only can EGFR activation increase tumor growth, PKM2 can also elevate EGF/EGFR activity [4]. Yang's group showed that EGFR activation could cause PKM2 translocation into the nucleus, where phosphorylation activates PKM2, which then interacts with β-catenin, leading to cyclin D1 expression, which is related with cancer cell proliferation [7]. These findings all demonstrate the important effects of PKM2 on human cancers.

However, the role of PKM2 in ovarian cancer is still poorly understood. To date, few investigations have focused on the relationship between PKM2 and ovarian cancer. Accordingly, we investigated its role in ovarian cancer in this study. We found that PKM2 was overexpressed in ovarian cancer tissues and that its upregulation was related to tumor size. Further research showed that PKM2 overexpression resulted in both elevated ovarian cancer cell proliferation, growth, and survival, which were related to overexpression contributing to increased cell cycle progression. Further data showed that the molecular mechanism of the function of PKM2 may be linked to its ability to change the expression levels of CCND1 and CDKN1A, which are 2 of the most widely demonstrated cell cycle regulators.

Conclusions

PKM2 is overexpressed in ovarian cancer tissue, and higher expression of PKM2 is related to larger tumor size as well as to increased ovarian cancer cell oncogenesis and development. Therefore, PKM2 may be a new treatment target in ovarian cancer.

Conflict of interest

None.

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References:

1. Cohen JG, White M, Cruz A, Farias-Eisner R: In 2014, can we do better than CA125 in the early detection of ovarian cancer? World J Biol Chem, 2014; 5: 286–300
2. Arboleda MJ, Lyons JF, Kabbinavar FF et al: Overexpression of AKT2/protein kinase Bbeta leads to up-regulation of beta1 integrins, increased invasion, and metastasis of human breast and ovarian cancer cells. Cancer Res, 2003; 63: 196–206
3. Goldberg MS, Sharp PA: Pyruvate kinase M2-specific siRNA induces apoptosis and tumor regression. J Exp Med, 2012; 209: 217–24
4. Hsu MC, Hung WC, Yamaguchi H et al: Extracellular PKM2 induces cancer proliferation by activating the EGFR signaling pathway. Am J Cancer Res, 2016; 6: 628–38
5. Singh H, Longo DL, Chabner BA: Improving prospects for targeting RAS. J Clin Oncol, 2015; 33: 3650–59
6. Sun Q, Chen X, Ma J et al: Mammalian target of rapamycin up-regulation of pyruvate kinase isoenzyme type M2 is critical for aerobic glycolysis and tumor growth. Proc Natl Acad Sci USA, 2011; 108: 4129–34
7. Yang W, Xia Y, Ji H et al: Nuclear PKM2 regulates beta-catenin transactivation upon EGFR activation. Nature, 2011; 480: 118–22
8. Liu W, Zhong S, Chen J, Yu Y: HER-2/neu overexpression is an independent prognostic factor for intestinal-type and early-stage gastric cancer patients. J Clin Gastroenterol, 2012; 46: e31–37
9. Huang Q, Lan F, Zheng Z et al: Akt2 kinase suppresses glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-mediated apoptosis in ovarian cancer cells via phosphorylating GAPDH at threonine 237 and decreasing its nuclear translocation. J Biol Chem, 2011; 286: 42211–20
10. Cazzalini O, Scovassi AI, Savio M et al: Multiple roles of the cell cycle inhibitor p21(CDKN1A) in the DNA damage response. Mutat Res, 2010; 704: 12–20
11. Liu JY, Qian D, He LR et al: PinX1 suppresses bladder urothelial carcinoma cell proliferation via the inhibition of telomerase activity and p16/cyclin D1 pathway. Mol Cancer, 2013; 12: 148
12. Ju X, Casimiro MC, Gormley M et al: Identification of a cyclin D1 network in prostate cancer that antagonizes epithelial-mesenchymal restraint. Cancer Res, 2014; 74: 508–19
13. Wu DW, Liu WS, Wang J et al: Reduced p21(WAF1/CIP1) via alteration of p53-DDX3 pathway is associated with poor relapse-free survival in early-stage human papillomavirus-associated lung cancer. Clin Cancer Res, 2011; 17: 1895–905
14. Christofk HR, Vander Heiden MG, Wu N et al: Pyruvate kinase M2 is a phosphotyrosine-binding protein. Nature, 2008; 452: 181–86
15. Eigenbrodt E, Basenau D, Holthusen S et al: Quantification of tumor type M2 pyruvate kinase (Tu M2-PK) in human carcinomas. Anticancer Res, 1997; 17: 3153–56
16. Oremek G, Kukshaite R, Sapoutzis N, Ziolkowski P: [The significance of TU M2-PK tumor marker for lung cancer diagnostics]. Klin Med (Mosk), 2007; 85: 56–58
17. Wechsel HW, Petri E, Bichler KH, Feil G: Marker for renal cell carcinoma (RCC): The dimeric form of pyruvate kinase type M2 (Tu M2-PK). Anticancer Res, 1999; 19: 2583–90
18. Vander Heiden MG, Locasale JW, Swanson KD et al: Evidence for an alternative glycolytic pathway in rapidly proliferating cells. Science, 2010; 329: 1492–99
19. Wong N, Ojo D, Yan J, Tang D: PKM2 contributes to cancer metabolism. Cancer Lett, 2015; 356: 184–91
20. Zhou CF, Li XB, Sun H et al: Pyruvate kinase type M2 is upregulated in colorectal cancer and promotes proliferation and migration of colon cancer cells. IUBMB Life, 2012; 64: 775–82
21. Kwon OH, Kang TW, Kim JH et al: Pyruvate kinase M2 promotes the growth of gastric cancer cells via regulation of Bcl-xL expression at transcriptional level. Biochem Biophys Res Commun, 2012; 423: 38–44
22. Liang J, Cao R, Zhang Y et al: PKM2 dephosphorylation by Cdc25A promotes the Warburg effect and tumorigenesis. Nat Commun, 2016; 7: 12431
23. Park SH, Ozden O, Liu G et al: SIRT2-mediated deacetylation and tetramerization of pyruvate kinase directs glycolysis and tumor growth. Cancer Res, 2016; 76: 3802–12
24. Park YS, Kim DJ, Koo H et al: AKT-induced PKM2 phosphorylation signals for IGF-1-stimulated cancer cell growth. Oncotarget, 2016; 7: 48155–67

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