Pyruvate Kinase M2 is Highly Expressed in Cutaneous Squamous Cell Carcinoma and Promotes its Progression

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Abstract

Background: Pyruvate kinase M2 (PKM2) is conducive to tumor formation and progression for several types of cancer. Its role in the tumorigenesis and progress of cutaneous squamous cell carcinoma (CSCC) remains unknown. We sought to explore the expression and function of PKM2 in CSCC.

Methods: The expression of PKM2 in 62 cases of actinic keratoses (AK), 15 cases of Bowen's diseases (BD), 66 cases of CSCC and 10 cases of normal skin tissues (NT) were detected by immunohistochemistry. The staining patterns were assessed using a 12-point semi-quantitative scoring system. Immunohistology scores were statistically analysed. We further established a stable PKM2 knockdown CSCC cell line to explore its effect on cell biological behaviors. In this study, we used western blotting, CCK-8 assay, colony formation test, transwell assay, wound-healing assay, glucose concentration assay and lactate concentration assay.

Results: We found that the expression of PKM2 increased significantly in AK, BD and CSCC, compared to that in NT. In addition, a comparison of PKM2 expression percentage showed that there was significantly difference among these three diseases. Then, we observed that PKM2 expression was associated with infiltration depth in 62 AK patient and course of disease in 15 BD patients. Moreover, high expression of PKM2 in 66 CSCC patients was significantly related with position, histological grade and Broder stage. In addition, PKM2 knockdown could obviously inhibit CSCC cell proliferation, invasion, migration and aerobic glycolysis in vitro. Finally, the expression levels of the phosphorylated- (p-) p38 of the MAPK pathway in the PKM2 knockdown groups greatly decreased compared with those of the controls in CSCC cell lines.

Conclusions: PKM2 is differently expressed in CSCC-related diseases. PKM2 is highly expressed in CSCC and promotes its progression, which may be associated with the activation of p38 MARK signaling pathway. PKM2 may be a potential therapeutic target for CSCC.

Background

Cutaneous squamous cell carcinoma (CSCC) is a primary nonmelanoma skin cancer which originates from epidermal keratinocytes^[1]. Compared with young people, middle-aged and elderly people are more likely to suffer from CSCC. Its occurrence is related to several risk factors, such as ultraviolet (UV) radiation, immunosuppression and genetic disorders^[2]. The incidence rates of CSCC continues to increase worldwide in recent years, particularly in aging countries^[3]. The CSCC development conforms to the classical multistep canceration model: precancerous lesions /Actinic keratosis (AK), squamous cell carcinoma in situ /Bowen disease (BD), invasive carcinoma and metastatic carcinoma. AK and BD are regarded as the precursors of invasive CSCC, and both of them can progress to CSCC^[4]. Surgical resection of early CSCC is an effective treatment. But, a few patients have surgical contraindications

which are still challenging to treat^[5]. Moreover, 5% of CSCC patients have lymph node metastasis, and some patients can even metastasize to visceral organs. The 10-year survival rate of patients with local lymphatic metastasis or distant metastasis is less than 20%^[6]. As such, it is essential to further explore the pathogenesis and the therapeutic targets of CSCC and develop the potential treatment for patients with CSCC.

Reprogramming of energy metabolism is one of the typical biological capabilities acquired by cancer during the multistep development of human tumors^[7]. Under oxygenated conditions, normal cells obtain energy mainly through the aerobic oxidation of glucose, but tumor cells mainly metabolize glucose by rapid glycolysis and produce a large amount of lactic acid. This metabolic reprogramming is called Warburg effect, also known as aerobic glycolysis^[8]. The whole process of glycolysis includes reversible enzymatic reactions controlled by many metabolic enzymes and irreversible enzymatic reactions controlled by three rate-limiting enzymes. Glucose metabolic reprogramming plays an important role in the malignant transformation of tumor, and the overexpression of glycolytic enzymes is closely related to tumor growth^[9].

Pyruvate kinase (PK) is the last rate-limiting enzyme in the glycolytic pathway, which catalyzes phosphoenolpyruvate to pyruvate and ATP. There are four isomeric forms of PKs: PKL, PKR, PKM1, and PKM2. PKM2 is highly expressed in many kinds of tumors and has two forms: high active tetramer and low active dimer^[10]. In tumor cells, the low active dimer predominates in tumor metabolism and contributes to tumorigenesis. The advantage of the low active dimeric form promotes the accumulation of phosphometabolites, enabling tumor cells to participate in high levels of synthetic processing to meet their energy and material needs^[11]. In addition, PKM2 can regulate the key signaling pathways to control cell diverse biological functions as a transcriptional coactivator^[12]. Therefore, PKM2 is currently believed to play an important role in tumor metabolism and progression.

Although PKM2 is regarded as a pivotal regulatory factor of glycolysis, and extensively participates in tumorigenesis, its expression and clinical significance in CSCC have not been reported. In this research, we explored PKM2 expression in human CSCC-related diseases, and evaluated its potential function for the malignant phenotype of CSCC cell lines.

Materials And Methods

Cases and specimen materials

A total of 62 AK, 15 BD, and 66 CSCC patients treated with surgical resection at the Department of Dermatology of Affiliated Hospital 2 of Nantong university, Nantong, China from 2014 to 2020 were recruited in this study. 10 cases of normal skin tissues (NT) were used as a normal control group. None of patients enrolled in this study received chemotherapy, radiotherapy, or immunotherapy before surgical resection. The diagnosis of postoperative specimens was pathologically confirmed. The pathological grades were classified into well-differentiated, moderately differentiated and poorly differentiated. The

Broder grades were classified into grade I, II, III and IV. In statistical analysis, moderately differentiated and poorly differentiated were classified as poorly differentiated. This study had been approved by the Second Affiliated Hospital of Nantong university Ethics Committee. All patients were informed with the whole operation protocol and signed informed consent.

Cell culture and reagents

CSCC cell lines (A431, Scl-1) were purchased from Cell Bank of the Chinese Academy of Science (Shanghai, China). All cell lines were cultured in DMEM medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco) in the atmosphere under 5% CO₂ at 37°C in a humidified incubator.

Immunohistochemistry (IHC)

IHC was performed in the total paraffin-embedded tissues to determine the intensity and proportion of PKM2 protein expression. All the samples were fixed with 4% paraformaldehyde and then embedded with paraffin. Serial sections of 4 µm were prepared for IHC. The tissue sections were deparaffinized and subjected to heat-induced antigen retrieval. Then, the sections were stained with a rabbit anti-human PKM2 mAb (1: 800;4053S, Cell Signaling Technology, Danvers, MA, USA) .

Scoring of PKM2 expression

Staining for PKM2 was scored based on a 12-point semi-quantitative scoring system^[12]. The positivity of the reaction was scored by counting the number of positive cells: 0%–25% (1), 26%–50% (2), 51%–75% (3), and ≥75% (4). The intensity was assessed as either negative staining (0), weakly positive (1), moderately positive (2), and strongly positive (3). The scores of staining intensity and proportion were multiplied for comprehensive evaluation, with a range from 0 to 12. The histological score was divided as follows: negative (-, 0-3 points), slightly positive (+, 4-6 points), positive (++ , 7-9 points) and strongly positive (+++, 10-12 points), while negative and weakly positive expression belong to low expression and the positive and strongly positive expression belong to high expression.

shRNA constructs and lentiviral production

Lentivirus-sh-PKM2 and control sh-NC were provided by Youxiweinan Biotechnology Company (Sanming, China). The shRNA with PKM2 specific exon 10 sequence knockdown were used (5'-CTGGCATCATCTGTACCAT-3'). Lentivirus was packaged using a three-plasmid system. ShRNAs were inserted into the doxycycline (DOX) -inducible gene expression vector Tet-pLKO.1-puro and cotransfected into 293T cells together with PSPAX2 and PMD2G. Lentivirus was harvested 36 h after transfection. A431 and Scl-1 cells were infected with lentivirus for 24 hours. Then, puromycin was used to select the stable transduction cell lines (0.8ug/ml for A431 cells, and 1.0 ug/ml for Scl-1 cells). Finally, dox was used at 1µg/ml for DOX-inducible transgene expression. Empty vector -transfected cells were used as the normal control (NC) cells. The cells were divided into 4 groups: NC group, NC+DOX group, shPKM2 group, shPKM2+DOX group.

Western blot analysis

Proteins for Western blot were extracted by RIPA buffer (Beyotime, Shanghai, China). Equal amounts of protein were first resolved by 10% SDS-PAGE gel and transferred to polyvinylidene difluoride membranes. The membranes were blocked at room temperature for 1 hour and then incubated with the primary antibodies (1: 1000) overnight at 4°C. GAPDH (1:5000, Proteintech, Wuhan, China) was used as the loading control. After washing the membranes with TBST for three times, the membranes were incubated with peroxidase-conjugated secondary antibodies (1:5000) at room temperature for 1 hour, and the signals were detected by Bio-rad chemiluminescence imaging system.

Cell proliferation and colony formation assays

For CCK-8 assays, the PKM2 knockdown cells or the negative control cells were seeded into 96-well plates at 2,000 cells/well. After 24, 48, 72 and 96 hours, the cell proliferation assay was determined by Cell Counting Kit-8 reagents (Proteintech, Wuhan, China). For the colony formation assay, after being digested by 0.25% trypsin, the cells were prepared into single cell suspension in DMEM medium containing 10% fetal bovine serum. The cell suspension was diluted in gradient and inoculated in a petri dish with an appropriate number of cells. Then, gently shake the petri dish to disperse the cells evenly and the cells were cultured in incubator for 1-2 weeks. The culture was terminated when a clone visible to the naked eye appeared in the petri dish. After paraformaldehyde fixation and crystal violet staining, the number of clones was calculated.

Transwell migration and invasion assays

A 24-well plate was equipped with Transwell chambers with a pore size of 8 mm. The cells at a density of 1.0×10^5 suspended in serum-free medium were seeded on the upper chambers. DMEM containing 30% FBS was added to the lower chamber. The cells were then incubated in a humidified incubator at 37°C for 24h. The extra medium was removed with absorbent paper, and the upper chamber cells were removed with a cotton swab. Next, the cells in the lower chamber were fixed with 4% paraformaldehyde for 15 min and stained with 0.1% crystal violet at room temperature for 30 min. Invasion assays were conducted in accordance with the above procedures except that the Transwell chambers were pretreated by Matrigel. A light microscope was used to count cells in randomly selected fields of view.

Wound healing assay

Cells were plated into 6-well plates at a density of 2.0×10^5 cells/well. When the cells reaching about 95% confluence, a 100- μ l pipette tip was used to scratch the cells. the non-adherent cells were removed by PBS. Subsequently, the cells were cultured in serum-free DMEM in an incubator at 37°C. At 0, 24 and 48h, an inverted microscope was used to collect the images. The healing rate were analyzed by ImageJ software (NIH, Bethesda, USA).

Glucose and lactate concentration assay

Cells at a density of 1.0×10^5 were seeded into 6-well plates with 2.5ml 10% FBS DMEM complete medium. The supernatants were collected at 24h, 48h and 72h respectively and stored at -80°C for glucose and lactate concentration assay. Glucose and lactate concentration were quantified using

GlucoseAssay Kit (Rsbio, Shanghai,China) and (Jiancheng, Nanjing, China)according to the standard protocols.

Statistical analysis

SPSS 25.0 statistical software and Graph-Pad Prism 8 software were used for the statistical analyses. The normality of all data distributions was assessed by the Kolmogorov-Smirnov test. Student's t test was selected to assess normally distributed parameter; otherwise, Mann- Whitney U test was performed. The chi-square test was used for categorical variables. P-values less than 0.05 were considered as statistically significant difference.

Results

Higher expression of PKM2 in CSCC-related diseases than normal controls

To investigate the expression of PKM2 in CSCC-related diseases, we conducted IHC on AK, BD, CSCC and NT specimens. IHC revealed positive staining of PKM2 mainly in the nucleus (light yellow to sepia colour), and partly in the cytoplasm (Fig. 1A). The rate of high positive expression of PKM2 was higher in the CSCC-related diseases specimens than that of the NT specimens, with different staining intensities. And the observed IHC score ranged from 1 to 12 points (Fig. 1B,C,E).

Correlation of PKM2 expression and clinicopathological features in CSCC-related diseases

To investigate the clinicopathological relevance of PKM2 expression in CSCC-related diseases, the patients were assigned to high or low expression groups based on their IHC scores (high expression groups: IHC score<7, low expression groups: IHC score≥7). PKM2 expression was associated with infiltration depth in 62 AK patients, and was associated with course of disease in 15 BD patients ($P < 0.05$,Table 1). In 66 CSCC patients, PKM2 expression was significantly associated with tumor position, histological grade and Broder stage ($P < 0.05$ Table 2). There was no significant relationship between PKM2 expression and other clinicopathological characteristics of CSCC, including gender, age, course of disease and tumor diameter (Table 2).

Table 1. Association between PKM2 expression and clinicopathological features of 62 AK patients and 15 BD patients

Characteristic	PKM2 expression in AK				PKM2 expression in BD			
	Low	High	χ^2	<i>P</i> -value	Low	High	χ^2	<i>P</i> -value
Gender			0.452	0.583			3.636	0.103
Male	12	9			0	4		
Female	27	14			6	5		
Age			0.637	0.675			2.784	0.235
≤70	3	1			3	1		
>70	29	11			3	8		
Position			0.147	1.000			0.069	0.569
Exposure	38	22			1	2		
Unexposed	1	1			5	7		
Course of disease			0.777	0.540			6.667	0.028*
≤2 years	31	16			6	3		
>2 years	8	7			0	6		
Infiltration depth(mm)			21.239	0.000*			0.045	1.000
≤1	33	6			3	5		
>1	6	17			3	4		
Diameter (cm)			0.084	0.793			1.111	0.525
≤1	15	8			2	1		
>1	24	15			4	8		

Table 2. Association between PKM2 expression and clinicopathological features of 66 CSCC patients

Characteristic	PKM2 expression		χ^2	P-value
	Low	High		
Gender			1.114	0.340
Male	6	12		
Female	10	38		
Age			0.978	0.436
≤70	1	8		
>70	15	42		
Position			6.468	0.027*
Exposure	12	48		
Unexposed	4	2		
Course of disease			0.025	1.000
≤2 years	9	27		
>2 years	7	23		
Infiltration depth (mm)			1.038	0.362
≤4	13	34		
>4	3	16		
Diameter(cm)			0.041	1.000
≤2	11	33		
>2	5	17		
Histological Grade			25.787	0.000*
Well-differentiated	14	9		
Poorly differentiated	2	41		
Broder stage			10.495	0.001*
I-II	15	24		
III-IV	1	26		

Precancerous lesions (AK and BD cases) versus CSCC

The rate of high positive expression of PKM2 in AK, BD, CSCC and NT were 37.10%, 60.00%, 75.76% and 0% respectively. A comparison of PKM2 expression percentage showed that there was significantly

difference among AK, BD and CSCC groups ($p < 0.05$, Table 3). Intensity of PKM2 expression in AK, BD and CSCC was significantly higher than that of NT, and intensity of PKM2 expression in CSCC was significantly higher than that of AK (-: 1~3 IHC score, + : 4~6 IHC score, ++ : 7~9 IHC score, +++ : 10~12 IHC score) ($p < 0.05$). But the intensity of PKM2 expression in BD was not significantly different with that in AK and CSCC ($P > 0.05$, Table 4 Fig. 1D).

Table 3. The rate of high positive expression of PKM2 in CSCC-related diseases (%)

Group	PKM2 expression		Total	High expression rate	χ^2	P-value
	Low	High				
NT	10	0	10	0	33.138	0.000*
AK	39	23	62	37.10%		
BD	6	9	15	60.00%		
CSCC	16	50	66	75.76%		
Total	71	82	153	53.59%		

Table 4. The intensity of PKM2 expression in CSCC-related diseases

Group	PKM2 expression				Mean Rank
	-	+	++	+++	
NT	10	0	0	0	13.00
AK	10	29	10	13	68.06
BD	0	6	4	5	89.17
CSCC	5	11	30	20	92.33

PKM2 suppression decreased proliferation of CSCC cells

In order to further understand the role of PKM2 in CSCC cells, we generated stable PKM2 knockdown cells by transfection with the PKM2-specific shRNAs which specifically targeted exon 10 without interfering with PKM1 expression. We confirmed the knockdown efficacy of the transfected A431 cell and Scl-1 cell by Western blotting (Fig. 2A, B). Then, we used CCK-8 assays and colony formation to judge whether PKM2 knockdown work for proliferation of A431 cell and Scl-1 cell. The results of CCK-8 assays revealed that knocking down PKM2 expression significantly inhibited cell growth compared to the negative control group, indicating that knockdown of PKM2 could inhibit the proliferation of A431 and Scl-1 cells. Furthermore, colony formation showed the similar trend (Fig. 2C, D).

PKM2 knockdown inhibited the migration and invasion in CSCC cells

To interrogate whether PKM2 have an impact on migration and invasion of CSCC cells, we conducted transwell assay and wound healing assays. Transwell assay presented that PKM2 knockdown significantly inhibited the migration and invasion of CSCC cells (Fig. 3A, B). Similarly, the wound-healing assays revealed that the migration ability of the PKM2 knockdown cells was significantly reduced compared with that of control cells (Fig. 3C, D).

PKM2 silencing altered glycolysis in CSCC cells

To investigate the effect of PKM2 knockdown on glycolysis of CSCC cells, we measured the glucose consumption and lactate production of CSCC cells. There was higher glucose level in the culture media of PKM2 knockdown cells when compared with the control groups, indicating the inhibited glucose consumption in PKM2 knockdown cells (Fig. 4A). In addition, the lactate production was also inhibited when silencing PKM2 in CSCC cells (Fig. 4B).

PKM2 knockdown inhibited the p38 MARK signaling pathway

To investigate the potential molecular mechanism of PKM2 in CSCC, we detected the mitogen-activated protein kinases (MAPKs) signaling pathways, which is aberrantly activated in multiple tumor cells. Silencing PKM2 significantly inhibited the expression of phosphorylated p38 of in CSCC cells compared to the NC group. Meanwhile, the total p38 protein expression remained unchanged (Fig. 5A, B,C).

Discussion

CSCC is the second most frequent skin cancer globally, amount to 20–50% of skin cancers^[13]. Surgical excision is the first-line treatment for early CSCC, with the overall outstanding survival rates^[14]. However, advanced CSCC needs the combined treatment, including chemotherapy, radiotherapy and systemic therapy^[15, 16], but in general, these curative treatments are not effective. Patients with advanced CSCC still have higher mortality^[17, 18]. CSCC may be primary or the result of the progression of AK and BD^[19, 20]. We try to find out whether PKM2 is dysregulated in the early stage of CSCC formation by careful study of CSCC precancerous lesions (AK and BD). The occurrence and development of CSCC is a gradual process. And different stages have different pathological definitions, including histological grade and Broder stage. These pathological changes may be regulated by several oncogenes and tumor suppressor genes^[21]. At present, there are few studies on the basic pathobiological mechanism of the tumorigenesis and progression of CSCC. For this reason, The knowledge of these processes in CSCC is crucial to identify novel molecular targets for therapy.

Glucose metabolism reprogramming is the result of self-regulation of tumor cells. In order to meet the needs of tumor development, the majority of the tumor cells show a high glycolysis phenotype^[22, 23]. In recent years, more and more studies have shown that PKM2 serves an important role in tumor

progression and has exhibited both glycolytic or non-glycolytic functions^[24, 25]. PKM2 is highly expressed in various types of cancer, which can be used as an independent biomarker for diagnosis, prognosis, and treatment effect evaluation^[26]. Overexpression of PKM2 may promote malignant biological behaviors, such as cancer invasion, metastasis, chemotherapy tolerance and so on^[27, 28]. For example, Xiao et al. demonstrated that PKM2 was upregulated in breast cancer tissues and overexpression of PKM2 could encourage tumor metastasis by promoting Epithelial-mesenchymal Transition(EMT)^[29]. Furthermore, Li et al. reported that PKM2 was significantly upregulated in HCC and promoted the proliferation, migration and invasion of HCC cells in vitro and the growth of tumor in vivo through inducing immunosuppressive microenvironment^[30]. Besides that, Cheng et al. reported that PKM2 can stimulate the invasion and migration of cancer cells in pancreatic ductal adenocarcinoma by enhancing the binding of PAK2-HSP90 complex, and promote the phosphorylation and stabilization of PAK2 protein^[31]. The above studies have revealed an oncogenic role of PKM2 in tumorigenesis. However, the effect of PKM2 on CSCC has not been studied formerly. In the present study, we examined the effect of PKM2 knockdown on the metabolic and non-metabolic functions in CSCC cells.

The present study revealed that PKM2 is expressed in different degrees in AK, BD and CSCC. The rate of high positive expression of PKM2 in CSCC was significantly higher than that in AK, BD and NT. Considering both of AK and BD is possible to develop into CSCC, this indicated that increased PKM2 expression is associated with CSCC tumorigenesis. Meanwhile, Dayton et al. found that the loss of PKM2 at the beginning of tumor caused delayed tumorigenesis and decreased tumor penetrance in soft tissue sarcoma, which meant that PKM2 expression may facilitate onset of this tumor type^[32]. The chi-square test showed that PKM2 high expression had close association with infiltration depth in AK and course of disease in BD. This indicated that the expression of PKM2 increased with the progression and duration of the disease.

Besides, we found the PKM2 high expression is closely related to position, histological grade and Broder stage. PKM2 was highly expressed in the exposed positions, which might be related to the progression of CSCC caused by UV damage. And the result showed that the expression of PKM2 was higher in stage III and stage IV CSCC tissue samples than that in stage I and stage II CSCC tissue samples. Moreover, intense PKM2 immunostaining was observed in poorly-differentiated of CSCC compared with that in well-differentiated of CSCC. Both of histological grade and Broder stage were related to the degree of malignancy. This meant that PKM2 had a significant effect on the invasive development of CSCC. Likewise, Long et al. detected the expression of PKM2 proteins in 74 lung adenocarcinoma cases and the corresponding noncancerous tissues by immunohistochemistry. They found that high expression of PKM2 in tumor cells was associated with both lymph node metastasis and TNM stage. The results suggested that PKM2 could enhance the malignancy of lung adenocarcinoma^[33]. Up to now, PKM2 has been proved to be interrelated to the degree of malignancy.

Finally, we found that PKM2 plays an important role in the cell biological behaviors of CSCC by using the cell lines A431 with PKM2 knockdown. Many studies have proved that PKM2 knockdown could decrease

carcinogenesis. For instance, In gastric carcinoma, PKM2 knockdown could inhibit the synthesis of E-cadherin and promote vimentin and N-cadherin expression. Thus, it could suppress cancer cell invasion, migration and the EMT phenotype^[34]. In the present study, we observed that the PKM2 knockdown cells drastically decreased their proliferation, which suggests a relationship between PKM2 overexpression and CSCC cell proliferation. Moreover, the knockdown of PKM2 reduced the migration and invasion rates of CSCC cells. Research on the metabolic function of PKM2, We found that PKM2 knockdown decreased glucose consumption and inhibited lactate production. In addition, Zhu et al. reported that the PKM2 knockdown can reduce the expression of MMP2 and MMP9, Thus blocking MAPK signaling pathway and inhibiting tumor cell invasion and migration^[35]. In this study, the result showed that the PKM2 knockdown inhibited the phosphorylation of p38, Therefore, we hypothesized that PKM2 promotes the phosphorylation of p38 to enhance the transcriptional activity and to promote cell proliferation, migration, and invasion.

Due to the sample size is small and there is no consensus on the interpretation of PKM2 immunohistochemical staining results, this may have an impact on the results. And we chose only one cell line in this study, which may cause biased results. Our future research will include more patients and cell lines to further confirm our conclusion.

In conclusion, PKM2 is differently expressed in AK, BD and CSCC. PKM2 is upregulated in CSCC and closely related to position, histological grade and Broder stage. Meanwhile, PKM2 promotes CSCC progression as an oncogene.

Abbreviations

PKM2

Pyruvate kinase M2, CSCC:Cutaneous squamous cell carcinoma, AK:Actinic keratosis, BD:Bowen's disease, NT:Normal tissue, p-p38:phosphorylated-p38, UV:Ultraviolet, MAPKs:Mitogen-activated protein kinases, EMT Epithelial-mesenchymal Transition.

Declarations

Ethics approval and consent to participate

Written informed consents were obtained from all participants, and this study was permitted by the Ethics Committee of the Second Affiliated Hospital of Nantong university.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

None.

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Authors' contributions

XM designed the study. NL, JS and LZ performed the data analyses. NL and XG drafted the manuscript. DZ supervised the study and revised the manuscript. All authors read and approved the final manuscript.

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Figures

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Figure 1

Immunohistochemistry was used to assess PKM2 expression in AK, BD, CSCC and NT tissues. A: PKM2 had different expression levels in CSCC-related diseases. B: High expression rate of PKM2 in different tissues. C: The staining intensity of tissues belonged to the negative (0), weakly (1), moderate (2), highly positive classes (3). D: The comparison of intensity of PKM2 expression among AK, BD, CSCC and NT tissues. E: The quantification of PKM2 IHC expression scores. * $P < 0.05$.

Figure 2

PKM2 Knockdown inhibited cell proliferation of CSCC. A and B: The knockdown efficiency of PKM2 by shRNA was measured in the A431 and Scl-1 cells by Western blot. GAPDH was used as a positive control. C: Cell Counting Kit 8 Assay evaluated the proliferation of the PKM2 knockdown cells and the control cells. D: Colony formation analysis of the PKM2 knockdown cells and the control cells.

Figure 3

PKM2 knockdown inhibited cell invasion and migration of CSCC. A and B: Cell migrations and invasions were measured by transwell assays. Results were quantitated and depicted in the bar graph for transwell assays. C and D: Wound healing assay was performed to compare the mobility and healing capacity of PKM2 silenced CSCC cell lines and control cells. Results were quantitated and depicted in the bar graph for wound healing assay.

Figure 4

Effect of PKM2 knockdown on glycolysis in CSCC cells. A: The glucose concentration in media of CSCC cells. B: The lactate concentration in media of CSCC cells. *P<0.05 compared with the control cells.

Figure 5

PKM2 knockdown decreased expression of p38 phosphorylation in CSCC cells. A: p38 and p-p38 expression were determined by western blotting. B and C: Results were quantitated and depicted in the bar graph to analyze p38 and p-p38. *P<0.05 compared with the control cells.