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Effects of Casein Kinase 2 Alpha Subunit on the Proliferation, Invasion and Migration of Pancreatic Cancer PANC-1 Cells via the PI3K/Akt/GSK-3β Pathway

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KEYWORDS Casein Kinase 2 Alpha Subunit. Invasion. Migration. Pancreatic Cancer. Proliferation

ABSTRACT The researchers aimed to evaluate the functions of casein kinase 2 alpha subunit (CK2a) expression silencing in influencing pancreatic cancer (PC) PANC-1 cells from the aspects of invasion, proliferation and migration. As PC-1, PC PANC-1 and SW1990 cells exhibited significantly reduced CK2a mRNA expression level in contrast to normal pancreatic cells HPDE6-C7, while PC tissues displayed raised expression level compared with adjacent tissues ($P<0.05$). CK2a mRNA expression had significant correlations with nerve infiltration, lymph node metastasis, tumor differentiation degree and tumor-node-metastasis stage. After interfering with the expression of CK2a in PANC-1 cells, p-PTEN had elevated expression, while p-Akt473, p-GSK-3β, p-c-Raf, p-Akt308, p-PDK1, Snail and Vimentin, β-catenin had significantly down-regulated expressions, impeding cell growth, decreasing the number of healing wounds and invading cells. Moreover, the transplanted tumors were inhibited in terms of growth. For PC PANC-1 cells, their metastasis, proliferation and invasion were modulated by CK2a probably through regulating the PI3K/Akt/GSK-3β pathway that targeted epithelial-mesenchymal transition.

INTRODUCTION

As a common gastrointestinal malignancy, pancreatic cancer (PC) accounts for 80-90 percent of all pancreatic tumors (Wang et al. 2020). Over 227,000 people die of PC every year in the world (Du et al. 2021). Due to no specific early-stage symptoms, PC is diagnosed in the middle or advanced stage for some patients. Regarding the metastasis or invasion, chemotherapy, surgery and radiotherapy produce unsatisfactory therapeutic effects, so PC patients manifest poor prognosis, with a survival rate lower than 10 percent within 1 year (Yu et al. 2019). For this reason, developing targeted therapeutic drugs is crucial to preventing PC progression.

Casein kinase 2 or II (CK2), a highly conserved messenger-independent serine-threonine protein kinase, is a heterogeneous tetramer comprising 2 regulatory subunits B plus 2 catalytic subunits (a/a') (Jung et al. 2019). Besides, CK2 has many functions, of which the phosphorylated substrates (over 300 types discovered so far) are diverse (Kim et al. 2019). CK2 is associated with cell growth, proliferation, differentiation, metastasis, apoptosis and invasion (Tang et al. 2020). It is highly expressed in diversified tumors (Vermorken et al. 2013; Hashimoto et al. 2018; Anjum et al. 2022). CK2 phosphorylates its substrates to facilitate tumor cell metastasis, proliferation and invasion and to suppress its apoptosis, exerting a crucial effect on tumor onset and progression (Tripathi et al. 2019). Kim et al. (2013) reported that in lung cancer A549 cell line, CK2 inhibitor CX-4945 regulated the entire epithelial-mesenchymal transition (EMT) process by suppressing Smad2/3, Twist, Snail and protein kinase B (Akt), impeded A549 cell migration together with invasion, and down-regulated matrix metalloproteinase 2 (MMP-2) and MMP-9 expressions. Additionally, Liu et al. (2016) denoted that through targeting the nuclear translocation and degradation of tumor suppressor gene BRMS1, CK2a facilitated lung cancer invasion and metastasis. Nevertheless, it is necessary to clarify the mechanism of CK2a for PC invasion and metastasis.

Objectives

CK2a expression was interfered in PC cells through small interfering ribonucleic acid (siRNA)

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technology, aiming to not only assess the effects on PC cells in terms of the migrating and invasive abilities but also preliminarily r probe into related mechanism.

MATERIAL AND METHODS

General Data

Approval for the present study has been obtained from the ethic committee of the hospital, and all patients have provided written informed consent. Totally 90 PC patients treated in the researchers' hospital from January 2019 to October 2019 were enrolled. Their medical records, surgically resected normal tissue specimens and PC tissue specimens were collected. The specimens collected were stored in liquid nitrogen. General clinical data included patients' gender, tumor size, age, tumor site, tumor-node-metastasis (TNM) stage, differentiation degree, nerve invasion and lymph node metastasis. These patients were aged 35-78 years old. In terms of the TNM stage, there were 18, 23, 30 and 19 cases of stage I, II, III and IV PC, respectively. The exclusion criteria involved 1) patients with heart, liver or kidney dysfunction, 2) those with immune system diseases, 3) those with other malignant tumors, or 4) pregnant or lactating females.

Measurement of CK2a Expression in PC Tissues and Cells by Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

TRIzol method with primers of CK2a (FP: 5'-GAGCCCTGGGACTGTCCCC-3', RP: 5'-AACCTGTCCTCAAAACCA-3') and β-actin (FP: 5'-GCCCATCTATGAGGGTTACGC-3', RP: 5'-GCTTTAGCCACGCTCGGTC-3') was adopted to extract total RNAs under such conditions as pre-denaturation (2 min at 75°C), and then denaturation (5 min at 90°C), annealing (60 s at 60°C) and extension (30 s at 72°C) for a total of 40 cycles. $2^{-\Delta\Delta Ct}$ method was employed to determine the relative expression levels. For each sample, the assay was independently repeated 3 times.

Materials

PC cell lines (SW1990, PANC-1 and AsPC-1) together with human normal pancreatic ductal epithelial cell line (HPDE6-C7) were provided by ATCC cell bank (USA). BALB/c nude mice offered by SPF (Beijing) Biotechnology Co., Ltd were selected [license number: SYXK (Beijing) 2019-0030]. Gibco (USA) was the supplier of Roswell Park Memorial Institute (RPMI) 1640 medium plus trypsin. Fetal bovine serum provided by Sijiqing Biotechnology Co., Ltd. (China) was applied. Lipofectamine™ 2000 transfection kit was from Invitrogen (USA). For glyceraldehyde-3-phosphate dehydrogenase (GAPDH), miR-32-5p, EZH2, and U6 primers, BGI (Shanghai, China) was the provider. Bicinchoninic acid (BCA) kit and ECL development reagent were bought from Beyotime Institute of Biotechnology (China). Antibodies against phosphatase and tensin homolog deleted on chromosome ten (PTEN), phosphorylated PTEN (p-PTEN), p-catenin, Akt, p-glycogen synthase kinase-3β (GSK-3β), p-Akt473, p-c-Raf, E-cadherin, p-Akt308, p-phosphoinositide-dependent kinase 1 (PDK1), MMP-2, Vimentin, β-actin, Snail and MMP-9 were manufactured by Cell Signaling Technology (USA). Secondary antibodies were provided by Beijing Bersee Science and Technology Co., Ltd. (China). Microplate reader was provided by Thermo Fisher Scientific (Shanghai, China). Refrigerated centrifuge and protein electrophoresis apparatus were bought from Beckman (USA) and Bio-Rad (USA), respectively.

Cell Culture and Grouping

Cell lines HPDE6-C7, SW1990, PANC-1 and AsPC-1 were subjected to routine culture (37°C and 5% CO_2) by virtue of RPMI 1640 medium containing 10 percent fetal bovine serum. The medium was replaced with fresh one every 2 days. When the density was 80 percent, the cells were subjected to digestion with trypsin and subculture.

Cell lines PANC-1 were assigned as control group (cells without transfection), siRNA-negative control (NC) group (cells undergoing siRNA-NC transfection), and siCK2a group (cells receiving siCK2a transfection). PANC-1 cells in the latter two groups were added with 100 μL of Lipofectamine 2000 mixed solution with a final plasmid concentration of 100 nmol/L in accordance with the instructions of Lipofectamine™ 2000. As for the control group, pure Lipofectamine 2000 solution was added into PANC-1 cells. Following 4 h of transfection, the medium was changed. At 24 h after transfection, the success of transfection was

determined by means of Western blotting in addition to qRT-PCR. shCK2a1028: sense: 5'-GATCCCA-G A A G A T T T A T A T G A C T A T T C A A - GAGCTFCTGA-3', and antisense: 5'-AGCTTCA-G A A G A T T T A T A T G A C T A T C T C T-TGAATAGTCATATAAAT CTTCTGG-3'.

Determination of Proliferation Ability of Cells by Methyl Thiazolyl Tetrazolium (MTT) Assay

The digestion and implantation in 96-well plate (1×10^3/well) of the cells were conducted at 24 h subsequent to transfection, and 3 replicates were set for each group. Next, after 24 h, 48 h, 72 h, and 96 h of separate culture, the cells were incubated with MTT solution (37°C, 4 h) and centrifuged, followed by discarding of the supernatant and addition of DMSO. Lastly, the absorbance at 450 nm was acquired via the microplate reader.

Detection of Migration Ability of Cells Through Would Healing Assay

Inoculation of PANC-1 cells was carried out in a 6-well plate as per the same grouping and culture conditions as those mentioned below. Following 48 h of culture, the cells after trypsinization were seeded into a new 6-well plate, where 3 replicates were set for each group. In the case of single-wall growth of cells, wounds were scratched through a pipette tip (200 μL), and then phosphate-buffered saline (PBS) was employed to rinse off the detached cells. Afterwards, serum-free medium was supplemented to the cells, and the wound width was observed and recorded using a microscope. Afterwards, following another 24 h of cell cultivation, the observation and recording of the wound width were implemented using the microscope. Rate of cell wound healing = (width of wound at 0 h – width of wound at 24 h)/width of wound at 0 h ×100 percent, representing the migration rate.

Detection of Invasion Ability of Cells Through Transwell Assay

The plate in the Transwell chamber was covered with a layer (about 50 μL) of Matrigel. Then the upper chamber was supplemented with each group of single-cell suspension cultivated with serum-free medium, and RPMI 1640 medium containing 10 percent fetal bovine serum was utilized for coating the lower chamber. Next, 24 h of incubator culture (5% CO_2, 37°C), PBS washing, formaldehyde fixation, crystal violet staining and observation under the microscope of the Transwell chamber were performed, and the cells passing through the membrane were counted.

Measurement of Expressions of p-PTEN, p-PDK1, p-Akt473, E-cadherin, p-Akt308, β-catenin, p-GSK-3β. MMP-2, p-c-Raf, Vimentin, Snail and MMP-9 through Western Blotting

PANC-1 cells were added with cell lysis buffer and then centrifuged to harvest the supernatant. Next, the BCA kit for protein assay was adopted to examine the protein concentration. Afterwards, proteins were subjected to quantitative denaturation as well as isolation through SDS-PAGE, followed by quick PVDF membrane transfer. Thereafter, Tris-buffered saline-Tween 20 (TBST) solution blended with 5 percent skimmed milk was employed for 2 h of membrane blocking at room temperature. Later, and with corresponding primary antibodies against p-PTEN, p-GSK-3β, p-Akt473, p-c-Raf, p-Akt308, Vimentin, p-PDK1, Snail, E-cadherin, β-catenin, MMP-2 and MMP-9 (1:2000) were utilized for overnight incubation of the membrane (4°C). After that, the membrane underwent 3 times of TBST washing, 2 h of room-temperature secondary antibody (1:10000) incubation, and color development with DAB chromogenic reagent in dark. The protein gray value was recorded and photographed using a gel imager (Bio-Rad, USA). Relative quantitative analysis was conducted, with GAPDH as the control.

Subcutaneous Tumor Formation Assay of Nude Mice

PANC-1 cells were taken from siCK2a group plus siRNA-NC group when entering the logarithmic growth phase after transfection, which were trypsinized, washed and resuspended to prepare a single-cell suspension using serum-free medium (5×10^7 cells/mL). Next, the suspension was injected subcutaneously into the left (siRNA-NC group) and right (siCK2a group) armpits of nude mice. Ultimately, the mice were killed following four weeks to remove the subcutaneously transplanted tumors, followed by normal saline rinsing prior to weighing.

Statistical Analysis

SPSS 22.0 software offered by IBM Inc., USA was applied to accomplish statistical analysis. ($\bar{x} \pm s$) plus independent *t* test were used for the measurement data. [n (%)] and χ^2 test were adopted to present and examine the numerical data. P<0.05 signified a difference with statistical significance.

RESULTS

CK2a Expression in PC Tissues as well as Normal Tissues in Periphery

According to the qRT-PCR results (Fig.1), CK2a mRNA expression was elevated overtly in PC tissues in contrast to in surrounding tissues (P<0.05).

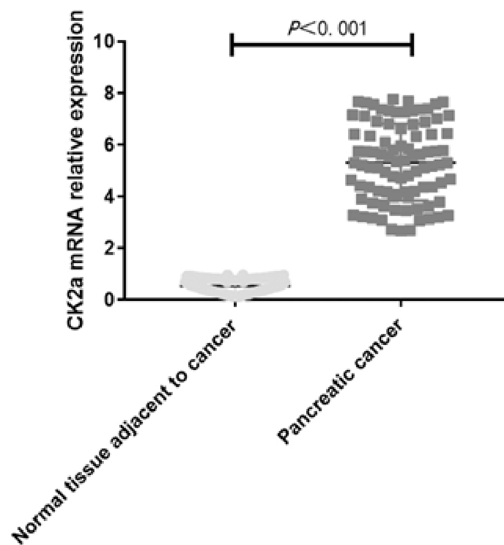

Fig. 1. CK2a mRNA expression in PC tissues as well as peripheral tissues detected via qRT-PCR. qRT-PCR: quantitative real-time polymerase chain reaction; CK2a: Casein kinase 2 alpha subunit; PC: pancreatic cancer

Relationships of CK2a mRNA Expression with Clinicopathological Factors of PC

The expression level of CK2a mRNA had no significant association with age, gender, tumor site or tumor size (P>0.05), whereas it presented prominent associations with TNM stage, nerve infiltration and tumor differentiation degree, in addition to lymph node metastasis. The patients with stage III-IV PC, nerve infiltration, lymph node metastasis, and moderate-poor differentiation had a higher mRNA expression level of CK2a (P<0.05) (Table 1).

Table 1: Relationships of CK2a mRNA expression with clinicopathological factors of PC

Factor	*n*	*Relative mRNA expression level of CK2a*	*P*
Age (year)			>0.05
<60	44	6.11±0.78	
>60	46	6.09±0.81	
Gender			>0.05
Male	52	6.14±0.83	
Female	38	6.08±0.76	
Tumor Site			>0.05
Head and neck	50	6.12±0.82	
Cauda	40	6.10±0.75	
Tumor Size (cm)			>0.05
<5	35	6.14±0.83	
>5	55	6.07±0.77	
Nerve Infiltration			<0.05
Yes	38	8.14±1.24	
No	52	5.32±0.54	
Differentiation Degree			<0.05
Poorly differentiated	47	9.19±1.43	
Moderately and highly differentiated	43	4.51±0.38	
TNM Stage			<0.05
I-II	41	4.21±0.41	
III-IV	49	10.32±1.89	
Lymph Node Metastasis			<0.05
Yes	42	12.34±1.22	
No	48	5.38±0.32	

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CK2a mRNA Expression Level in PC Cells

Normal pancreatic cells (HPDE6-C7) together with PC cell lines (SW1990, PANC-1 and AsPC-1) were selected to measure CK2a mRNA expression through qRT-PCR. Based on the results (Fig. 2), cell lines AsPC-1, PANC-1 and SW1990 had significantly increased mRNA expressions of CK2a in comparison with HPDE6-C7 cells, with the highest expression in PANC-1 cell line (P<0.05). Hence, PANC-1 cell line was utilized for subsequent assays.

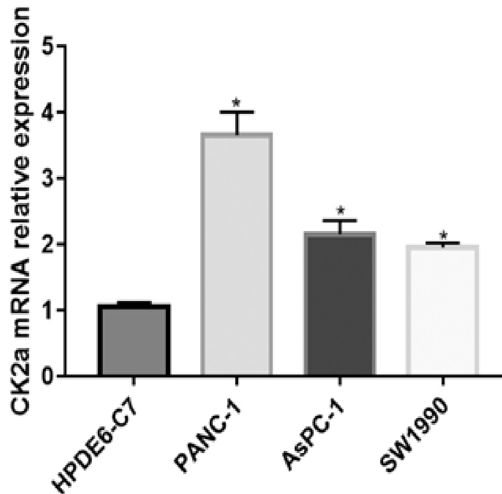


Fig. 2. Expression level of CK2a mRNA in PC cells determined using qRT-PCR. *P<0.05 vs. HPDE6-C7 cells. CK2a: Casein kinase 2 alpha subunit; qRT-PCR: quantitative real-time polymerase chain reaction; PC: pancreatic cancer

Function of Silenced CK2a in Influencing PANC-1 Cell Proliferation Ability

After silencing CK2a in PANC-1 cells, the transfection effect was checked by means of qRT-PCR. Compared to that in control and siRNA-NC groups, CK2a expression in the siCK2a group dropped dramatically (P<0.05) (Fig. 3A), implying the successful construction of cells with stably down-regulated CK2a.

As revealed by cell counting kit-8 (CCK-8) assay results in Figure 3B, the cell proliferation ability at 48, 72 and 96 h was conspicuously weaker in the siCK2a group than that in the siRNA-NC group (P<0.05). Therefore, silencing CK2a could apparently restrain the PANC-1 cell proliferation capability.

Impact of CK2a Silencing on PANC-1 Cell Invasive Capability

Following CK2a silencing, Transwell assay was executed to determine PANC-1 cell invasive capability. Compare with the siRNA-NC group, the siCK2a group had a significantly reduced number of cells in Matrigel (P<0.05) (Fig. 4). Thus, cell invasion ability was significantly weakened after silencing CK2a.

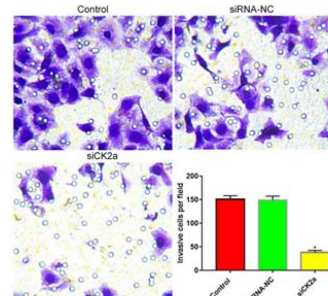
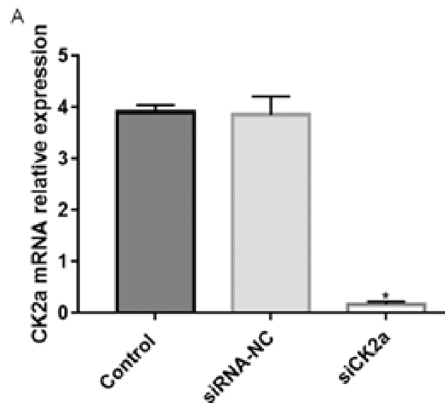


Fig. 4. Role of CK2a silencing in impacting PANC-1 cell invasion ability. *P<0.05 vs. siRNA-NC group. siRNA: small interfering ribonucleic acid; CK2a: Casein kinase 2 alpha subunit; NC: negative control



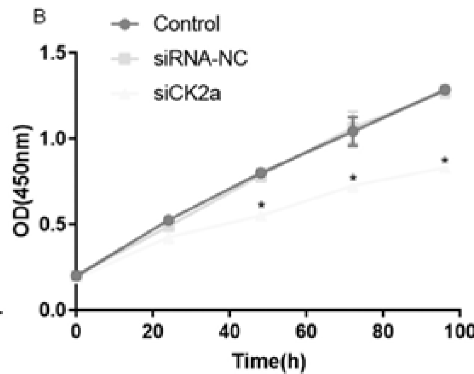


Fig. 3. Function of CK2a silencing in affecting PANC-1 cell proliferation ability. A: Effect of CK2a silencing inspected via qRT-PCR. *P<0.05 vs. siRNA-NC group. B: Proliferation capacity examined through CCK-8 assay. *P<0.05 vs. siRNA-NC group. NC: negative control; CCK-8: Cell counting kit-8; CK2a: casein kinase 2 alpha subunit; siRNA: small interfering ribonucleic acid; qRT-PCR: quantitative real-time polymerase chain reaction

Effect of CK2a Silencing on PANC-1 Cell Migrating Ability

Wound healing assay was applied to detect PANC-1 cell migrating ability with silenced CK2a. In contrast with the siRNA-NC group, the siCK2a group presented markedly reduced number of migrating cells plus rate of wound healing ($P<0.05$) (Fig. 5). Hence, cell migrating ability declined significantly after silencing CK2a.

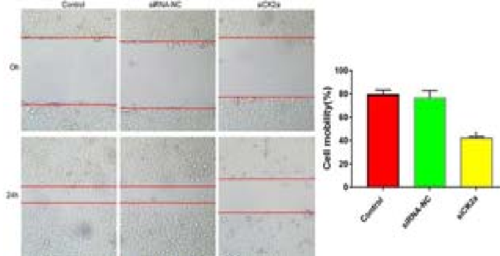


Fig. 5. Function of CK2a silencing in influencing PANC-1 cell migration ability. *P<0.05 vs. siRNA-NC group. CK2a: Casein kinase 2 alpha subunit; NC: negative control; siRNA: small interfering ribonucleic acid

Effects of CK2a Silencing on p-PTEN, p-GSK-3β,p-Akt473, p-c-Raf, p-Akt308, Vimentin, p-PDK1, Snail, E-cadherin, β-catenin, MMP-2 and MMP-9

According to Western blotting, in contrast to control and siRNA-NC groups, the siCK2a group had significantly elevated expressions of p-PTEN and E-cadherin and reduced expressions of p-Akt473, p-PDK1, p-Akt308, Vimentin, p-GSK-3β, Snail, p-c-Raf, β-catenin, MMP-2 and MMP-9 ($P<0.05$). Besides, the interference with CK2a expression up-regulated p-PTEN expression, reduced Akt activation to impede all downstream signaling events modulated by Akt, namely p-Akt473, p-PDK1, p-Akt308, Vimentin, p-GSK-3β-, β-catenin, pc-Raf and Snail, and down-regulated MMP-2 and MMP-9 expressions (Fig. 6). Collectively, interfering with CK2a expression not only suppressed the phosphatidylinositol 3-kinase (PI3K)/Akt/GSK-3β/ Snail signaling pathway activation besides MMP-9, but also restricted cell proliferation, invasion and metastasis.

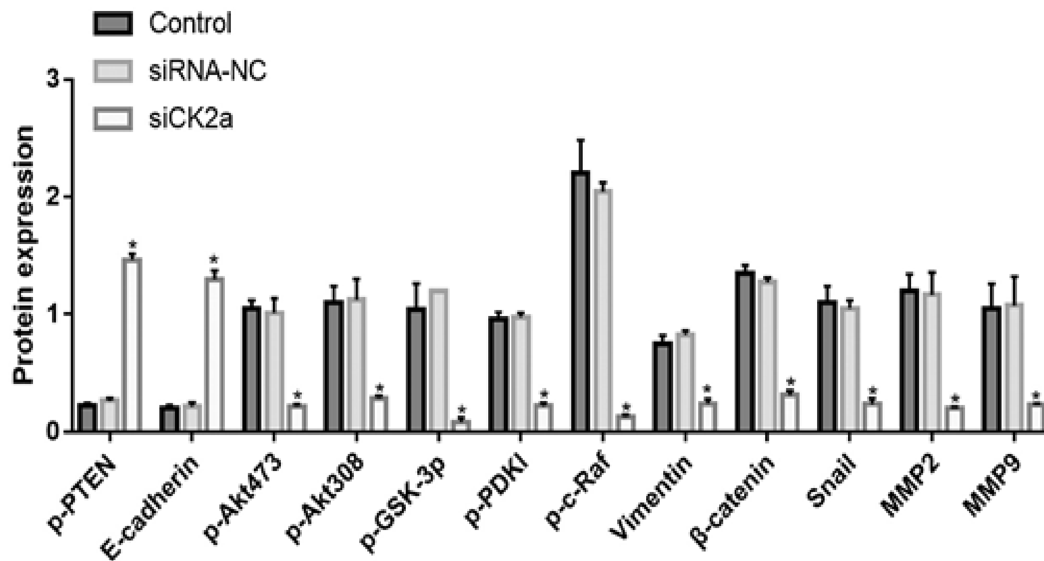


Fig. 6. Effects of CK2a silencing on p-PTEN, p-PDK1, p-Akt473, p-c-Raf, p-Akt308, Vimentin, p-GSK-3β, Snail, E-cadherin, MMP-9 β-catenin and MMP-2. *P<0.05 vs. siRNA-NC group. MMP: matrix metalloproteinase; CK2a: Casein kinase 2 alpha subunit; GSK-3β: glycogen synthase kinase-3β; NC: negative control; PDK1: phosphoinositide-dependent kinase 1; PTEN: phosphatase and tensin homolog deleted on chromosome ten; p-PTEN: phosphorylated PTEN; siRNA: small interfering ribonucleic acid

Effect of CK2a Silencing on Nude Mice in Terms of Subcutaneous Tumor Formation

Following lentivirus transfection, PANC-1 cells acquired from siRNA-NC and siCK2a groups were prepared into tumor single-cell suspensions. Then the nude mice were administered with the suspensions through subcutaneous injection into either left (siRNA-NC group) or right (siCK2a group) armpit to observe the function of silencing CK2a in affecting subcutaneous tumor formation. The siCK2a group exhibited notably lighter tumors than the siRNA-NC group (P<0.05) (Fig. 7).

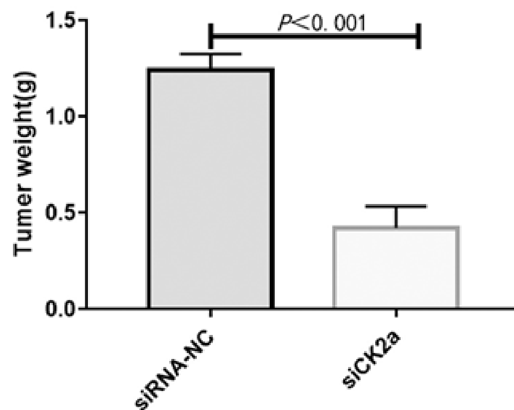


Fig. 7. Function of CK2a silencing in affecting nude mice from the aspect of subcutaneous tumorigenesis. *P<0.05 vs. siRNA-NC group. NC: negative control; CK2a: Casein kinase 2 alpha subunit; siRNA: small interfering ribonucleic acid

DISCUSSION

The invasion and metastasis of PC are complex and regulated by multiple oncogenes, tumor suppressor genes and many signaling pathways (Li et al. 2021). Thus, exploring the invasion and metastasis of PC cells is crucial for guiding the diagnosis, treatment and prognostic evaluation of PC. CK2 is a multifunctional protein kinase with diverse phosphorylation substrates, affecting cell growth, differentiation, proliferation, as well as apoptosis. Recently, it is involved in metastasis besides invasion of tumor, while the related mechanism for PC remains unclear. In this study, high expression of CK2a was obtained from PC PANC-1, SW1990 and AsPC-1 cells, and significantly related to nerve infiltration, lymph node metastasis, tumor differentiation degree, and TNM stage. Moreover, CK2a expression rose in stage III-IV PC patients with nerve infiltration, lymph node metastasis and moderate-poor differentiation (P<0.05), demonstrating that CK2a worked as a tumor-promoting gene.

After interference with the expression of CK2a in this study, PC PANC-1 cell invasive and migrating abilities were significantly weakened. Additionally, MMP-2 plus MMP-9 protein expressions also dropped remarkably. The results of *in vivo* experiments proved that silencing CK2a influenced the nude mice through inhibiting subcutaneous tumor formation. Taken together, CK2a exerted effects on PC migration, proliferation, as well as invasion.

During EMT, transformation of polar epithelial cells into active mesenchymal cells occurs, which are able to move freely between cell matrices (Roshan et al. 2019). Increasing evidence has confirmed that EMT is a crucial player in tumor metastasis and invasion (Li et al. 2016). In this study, Western blotting was employed to investigate the EMT-related protein expressions in PC after interference with the expression of CK2a. The results revealed that E-cadherin protein, an epithelial molecular marker, had an increased expression, whereas Vimentin and β-catenin, mesenchymal molecular markers, as well as the transcription factor Snail, had reduced expressions, suggesting that CK2a participated in PC invasion, proliferation and migration through EMT.

Being essentially implicated in modulating many important biological processes, the PI3K/Akt signaling pathway interacts with CK2 (Zhou et al. 2022). For instance, Ryu et al. (2012) found that in human prostate cancer LNCaP cell line, CX4945, a CK2 inhibitor, resisted androgen receptor activity and exerted an antitumor effect by suppressing the Akt-survivin signaling pathway. Recently, the PI3K/Akt signaling pathway has been reported to modulate EMT to expedite tumor invasion and metastasis. For example, in the report of Kim et al. (2021), the PI3K/Akt signaling pathway was involved in triggering EMT in squamous cell carcinoma cells and enhanced tumor cells from the angle of invading and metastasizing abilities. Nonetheless, the mechanism by which the PI3K/Akt signaling pathway regulates EMT retains unknown.

GSK-3β, a downstream gene of the PI3K/Akt signaling pathway, is able to regulate EMT by phosphorylating the transcription factor Snail. Li et al. (2016) found that OLAl regulated EMT by means of GSK-3β/Snail/E-cadherin in lung cancer,

thus controlling the disease via its invasion and metastasis. In breast cancer and gastric cancer, the PI3K/Akt/GSK-3β signaling pathway has a regulatory effect on EMT (Dai et al. 2016; Zhang et al. 2017). Thus, GSK-3β regulates EMT through the phosphorylation of Snail, thereby participating in tumor invasion and metastasis (Bai et al. 2016). After CK2a expression in PC PANC-1 cells in the present study was subjected to interference, p-PTEN expression rose, while the expressions of p-Akt473, p-PDK1, p-Akt308, Vimentin, p-GSK-3β, Snail, p-c-Raf and β-catenin significantly decreased. Accordingly, CK2a may modulate EMT by regulating Snail via the PI3K/Akt/GSK-3β signaling pathway, thus impacting PANC-1 cell invading and metastasizing capabilities.

CONCLUSION

In conclusion, the regulatory association of the PI3K/Akt signaling pathway with EMT is complicated. CK2a modulates EMT probably by regulating Snail via the PI3K/Akt/GSK-3β signaling pathway. Hence, CK2a regulates PC PANC-1 cell metastasis, proliferation and invasion through this pathway.

RECOMMENDATIONS

Further experiments should be carried out to verify whether CK2a regulates EMT through other potential signaling pathways.

ABBREVIATIONS

BCA: Bicinchoninic acid;
CCK-8: cell counting kit-8;
CK2a: Casein kinase 2 alpha subunit;
GAPDH: glyceraldehyde-3-phosphate dehydrogenase;
GSK-3β: glycogen synthase kinase-3β;
MMP: matrix metalloproteinase;
MTT: methyl thiazolyl tetrazolium;
NC: negative control; PBS: phosphate-buffered saline;
PC: pancreatic cancer;
PDK1: phosphoinositide-dependent kinase 1;
PTEN: phosphatase and tensin homolog deleted on chromosome ten;
p-PTEN: phosphorylated PTEN;
qRT-PCR: quantitative real-time polymerase chain reaction;
RPMI: Roswell Park Memorial Institute;
siRNA: small interfering ribonucleic acid;
TBST: Tris-buffered saline-Tween 20.

REFERENCES

Anjum F, Sulaimani MN, Shafie A et al. 2022. Bioactive phytoconstituents as potent inhibitors of casein kinase-2: Dual implications in cancer and COVID-19 therapeutics. *RSC Adv,* 12: 7872-7882.

Bai L, Yu Z, Zhang J et al. 2016. OLA1 contributes to epithelial-mesenchymal transition in lung cancer by modulating the GSK3β/snail/E-cadherin signaling. *Oncotarget,* 7: 10402-10413.

Dai J, Qian C, Su M et al. 2016. Gastrokine-2 suppresses epithelial mesenchymal transition through PI3K/AKT/GSK3β signaling in gastric cancer. *Tumour Biol,* 37: 12403-12410.

Du W, Lei C, Wang Y et al. 2021. LINC01232 sponges multiple miRNAs and its clinical significance in pancreatic adeno carcinoma diagnosis and prognosis. *Technol Cancer Res Treat,* 20: 1533033820988525.

Hashimoto A, Gao C, Mastio J et al. 2018. Inhibition of casein kinase 2 disrupts differentiation of myeloid cells in cancer and enhances the efficacy of immunotherapy in MiceCK2 inhibitor and MDSC. *Cancer Res,* 78: 5644-5655.

Jung M, Park KH, Kim HM et al. 2019. Inhibiting casein kinase 2 overcomes paclitaxel resistance in gastric cancer. *Gastric Cancer,* 22: 1153-1163.

Kim HJ, Kim DY, Cheon HG 2019. Clomiphene promotes browning of white adipocytes via casein kinase-2 inhibition. *Eur J Pharmacol,* 861: 172596.

Kim J, Hwan Kim S 2013. CK2 inhibitor CX-4945 blocks TGF-β1-induced epithelial-to-mesenchymal transition in A549 human lung adenocarcinoma cells. *PLoS One,* 8: e74342.

Kim J, Jang G, Sim SH et al. 2021. *SMARCA4* depletion induces cisplatin resistance by activating YAP1-mediated epithelial-to-mesenchymal transition in triple-negative breast cancer. *Cancers (Basel),* 13: 5474.

Li H, Peng C, Zhu C et al. 2021. Hypoxia promotes the metastasis of pancreatic cancer through regulating NOX4/KDM5A-mediated histone methylation modification changes in a HIF1A-independent manner. *Clin Epigenetics,* 13: 18.

Li SP, Xu HX, Yu Y et al. 2016. LncRNA HULC enhances epithelial-mesenchymal transition to promote tumorigenesis and metastasis of hepatocellular carcinoma via the miR-200a-3p/ZEB1 signaling pathway. *Oncotarget,* 7: 42431-42446.

Liu Y, Amin EB, Mayo MW et al. 2016. CK2α' drives lung cancer metastasis by targeting BRMS1 nuclear export and degradation. *Cancer Res,* 76: 2675-2686.

Roshan MK, Soltani A, Soleimani A et al. 2019. Role of AKT and mTOR signaling pathways in the induction of epithelial-mesenchymal transition (EMT) process. *Biochimie,* 165: 229-234.

Ryu BJ, Baek SH, Kim J et al. 2012. Anti-androgen receptor activity of apoptotic CK2 inhibitor CX4945 in human prostate cancer LNCap cells. *Bioorg Med Chem Lett,* 22: 5470-5474.

Tang S, Yuan Y, Liu Z et al. 2020. Casein kinase 2 inhibitor CX-4945 elicits an anti-Warburg effects through the down-regulation of TAp73 and inhibits gastric tumorigenesis. *Biochem Biophys Res Commun,* 530: 686-691.

Tripathi V, Chatterjee KS, Das R 2019. Casein kinase-2–mediated phosphorylation increases the SUMO-dependent activity of the cytomegalovirus transactivator IE2. *J Biol Chem,* 294: 14546-14561.

Vermorken JB, Stöhlmacher-Williams J, Davidenko I et al. 2013. Cisplatin and fluorouracil with or without panitumumab in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck (SPECTRUM): An open-label phase 3 randomised trial. *Lancet Oncol,* 14: 697-710.

Wang C, Huang Y, Zhang J et al. 2020. MiRNA 339 5p suppresses the malignant development of gastric cancer via targeting ALKBH1. *Exp Mol Pathol,* 115: 104449.

Yu Z, Zhao S, Wang L et al. 2019. MiRNA 339 5p plays an important role in invasion and migration of pancreatic cancer cells. *Med Sci Monit,* 25: 7509 7517.

Zhang X, Jiang G, Sun M et al. 2017. Cytosolic THUMPD1 promotes breast cancer cells invasion and metastasis via the AKT-GSK3-Snail pathway. *Oncotarget,* 8: 13357-13366.

Zhou L, Wang H, Liu H et al. 2022. The synergistic therapeutic effect of imatinib and protein kinase CK2 Inhibition correlates with PI3K-AKT activation in gastrointestinal stromal tumors. *Clin Res Hepatol Gastroenterol*, 46: 101886.

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