Inhibition of MARK2 inhibits ovarian cancer cell proliferation by regulating PI3K/AKT/p53 axis

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Abstract

Ovarian cancer (OC) is the 3rd most common type of the gynecological malignancy. Although current treatment strategies have greatly improved, there is still a need to develop new biomarkers for OC diagnosis and treatment. Microtubule affinity regulated kinase 2 (MARK2) is a kinase involved in the progression of multiple tumors. However, whether abnormal expression of MARK2 is associated with OC progression needs further analysis. We here revealed its role in OC. We found high expression of MARK2 in OC. Knockdown of MARK2 inhibited proliferation of OC cells, stimulated apoptosis of OC cells, and restrained glucose metabolism of OC cells. Furthermore, MARK2 regulated phosphatidylinositol 3-kinase/PKB (protein kinase B)/tumor suppressor protein 53 (PI3K/AKT/p53) axis in OC, therefore affecting the progression of OC. In summary, MARK2 knockdown suppressed cell proliferation by regulating PI3K/AKT/p53 axis.

Keywords

Ovarian cancer (OC); Microtubule affinity regulated kinase 2 (MARK2); Apoptosis; glucose metabolism; PI3K/AKT/p53

1. Introduction

Ovarian cancer (OC) is the 3rd most common gynecological type of malignancy worldwide [1]. OC is a heterogeneous malignancy with genomic features [2]. The incidence and mortality of OC is at 1.6% and 2.1%, respectively [3]. OC remains one of the most challenging types of malignancies [2]. At present, the clinical treatment of OC is mainly surgery as well as chemotheraphy, but targeted therapy is also can be selected [1, 4]. Targeted therapy generally includes two types, poly-ADP-ribose polymerase (PARP) inhibitors and anti-angiogenesis drugs, which can be applied to the first-line maintenance therapy for advanced ovarian cancer with limited efficacy [5]. Although current treatment strategies have been improved, new biomarkers are still needed to explore new therapeutic targets.

Microtubule affinity regulated kinase 2 (MARK2) is a kinase that is critical for neural differentiation, and cell polarization [6]. MARK2 regulates the activity of microtubule related proteins and regulates axon growth [7]. In addition, MARK2 is present at centrosomes and retraction fibers corrects spindle off-centering [8]. Recent studies have found that MARK2 was highly expressed in several tumor tissues and affected the progression of multiple tumors [9, 10]. MARK2 enhances cisplatin resistance in osteosarcoma cells through the PI3K/AKT/nuclear factor kappa-B (NF-κB) axis [11]. MARK2 enhances aerobic glycolytic-mediated breast cancer cell growth by modulating the target protein of rapamycin/Hypoxia-inducing factor 1α (mTOR/HIF-1α) as well as p53 pathways [12]. MARK2 promotes Warburg effect as well as cell growth in lung carcinoma [13]. Moreover, MARK2 affected the progression of multiple types of cancers, such as breast cancer [12]. In addition to the role of MARK2 in multiple tumors, its expression in OC has been previously described. Analysis of The Cancer Genome Atlas (TCGA) data through an online platform found that MARK2 transcription levels were higher in OC than in normal tissues. However, the possible effects of MARK2 on OC progression and the potential mechanism needs further study.

The aim of this study was to clarify the role of MARK2 in OC and explore the mechanism. This study confirmed that MARK2 was highly expressed in OC and MARK2 knockdown inhibited the proliferation and glucose metabolism of OC cells, and induced apoptosis of OC cells via the PI3K/AKT/p53 axis.

2. Materials and methods

2.1 Bioinformatics

Transcriptome data were obtained from The Cancer Genome Atlas databases. In addition, the expression level of MARK2 in OC were analyzed using Gene Expression Profiling Interactive Analysis (GEPIA) online platforms based on TCGA database.

2.2 Cell culture and transfection

Human ovarian epithelial cell line IOSE80, and 3 OC cell lines, including SK-OV-3, A2780 and OVCAR4 cells were all purchased from the Cell Bank of Chinese Academy of
Cells were cultured with the complete medium (DMEM medium containing 10% Fetal Bovine Serum (FBS, Gibco, CA, USA). After 12 hours of culture, Lipoctamine® 3000 reagent (L3000015, Invitrogen, Carlsbad, CA, USA) was used to transfect sh-negative control (NC), sh-MARK2 #1 and sh-MARK2 #2, respectively. 100 µL of the mixture was slowly added to the 6-well plate.

2.3 Quantitative Polymerase Chain Reaction (qPCR)
qPCR was performed with the SYBR-Green Master Mix (Roche, Basel, Switzerland) and respective primers. The sequences of primers: MARK2: F: 5'-CATATTGGAAACTACCGGCTC-3', R: 5'-GGAGGAGTTCAGTTGAGTCTTGTG-3', glyceraldehyde-3-phosphate dehydrogenase (GAPDH): F: AGAAGGCTGGGGCTCATTTG, R: AGGGGCCATCCACAGTCTTC'.

2.4 Western blotting
Total proteins were extracted from cells using Radio Immunoprecipitation Assay (RIPA) buffer (P0013K, Beyotime, Beijing, China), and the concentration of protein was quantitated by bovine serum albumin (BCA) reagent. Equal amounts of proteins were separated by Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE), and further transferred to Polyvinylidenefluoride (PVDF) membrane. The membranes were blocked with 5% milk and further transferred to Polyvinylidenefluoride (PVDF) membrane. The membranes were blocked with 5% milk for 1 h, and then the corresponding primary antibodies were added and incubated at 4 °C overnight. Primary antibodies included anti-MARK2 (Abcam, ab133724; 1:500, Cambridge, UK), Bax (Abcam, ab32503; 1:500, Cambridge, UK), Cleaved caspase 3 (Abcam, ab32042; 1:1000, Cambridge, UK), β-actin (Abcam, ab8226; 1:3000, Cambridge, UK), p-AKT (Abcam, ab38449; 1:1000, Cambridge, UK), AKT (Abcam, ab89592; 1:1000, Cambridge, UK), p-PI3K (Abcam, ab115730; 1:1000, Cambridge, UK), PI3K (Abcam, ab278545; 1:500, Cambridge, UK), Glucose transporter 1 (GLUT1) (Abcam, ab115730; 1:1000, Cambridge, UK), P53 (Abcam, ab302958; 1:1000, Cambridge, UK), p-PI3K (Abcam, ab278545; 1:500, Cambridge, UK), AKT (Abcam, ab8805; 1:1000, Cambridge, UK), p-AKT (Abcam, ab38449; 1:1000, Cambridge, UK), p53 (Abcam, ab26; 1:1000, Cambridge, UK), β-actin (Abcam, ab8226; 1:3000, Cambridge, UK). Then membranes were incubated with secondary antibodies for 1 h and photographed after chemiluminescence. The reagents used in this experiment were purchased from Wuhan Google Co., LTD.

2.5 CCK-8 assay
OC cells were maintained for 48 h. Cells were subsequently incubated with CCK-8 (C0038, Beyotime, Beijing, China) for 4 h. Then the absorbance value was measured at 450 nm wavelength.

2.6 Colony formation assay
Cells were plated into the 6-well plates and maintained in media (10% FBS) for 10 days at 37 °C. Then cells were fixed with paraformaldehyde (PFA) for 15 min and stained with 0.1% crystal violet for 20 min. Then the colonies were counted and photographed.

2.7 Cell apoptosis assay
The cells were fixed using 70% ethanol at −20 °C for 2 h. Cells were stained with propyl iodide (PI) and Annexin V at 4 °C and the cells at different phases were measured and analysed using FACSCalibur flow cytometer and CellQuest Pro 5.1 (342973, BD Biosciences, Inc., Franklin Lake, NJ, USA).

2.8 Glucose intake and lactate production tests
The glycolysis of cells was detected according to the glucose intake (ab136955, Abcam, Cambridge, UK) and lactate production (ab65330, Abcam, Cambridge, UK).

2.9 Statistics
GraphPad 5.0 software (National Institutes of Health, Bethesda, MD, USA) was used and performed for the statistical analysis. Data were represented as mean ± Standard deviation (SD), and p < 0.05 was thought as statistically significant.

3. Results

3.1 High expression of MARK2 in OC cells
To investigate the role of MARK2 in the progression of OC, we detected its expression levels in GEPIA database through bioinformation analysis. TCGA database analysis indicated the high transcript per million of MARK2 in OC tissues (Fig. 1A). Consistently, the mRNA and protein levels of MARK2 were detected in human ovarian epithelial cell line IOSE80, and 3 OC cell lines, including SK-OV-3, A2780 and OVCAR4 cells though qPCR and Immunoblot assays. The results confirmed the high expression of MARK2 in OC cells (Fig. 1B,C). Therefore, MARK2 was highly expressed in human OC.

3.2 The depletion of MARK2 inhibited proliferation of OC cells
The MARK2 shRNA plasmids shMARK2 #1 and shMARK2 #2 were transfected into OC cell lines, including SK-OV-3 and A2780 cells. Through Immunoblot assays, the transfection of MARK2 shRNA plasmids obviously decreased its expression (Fig. 2A). Through CCK-8 assays, the downregulation of MARK2 suppressed cell viability, with the decreased OD450 value (Fig. 2B). In addition, through colony formation assays, the downregulation of MARK2 decreased the colony numbers of SK-OV-3 and A2780 cells (Fig. 2C). These results suggested that MARK2 inhibited proliferation of OC cells.

3.3 Knockdown of MARK2 induced apoptosis of OC cells
We then investigated whether MARK2 affected the apoptosis of OC cells through Flow cytometry (FCM) assays. Interestingly, the downregulation of MARK2 increased the apoptosis percentage of SK-OV-3 as well as A2780 cells (Fig. 3A). Further, we noticed that MARK2 ablation increased the expression of cleaved caspase 3 as well as Bax in SK-OV-3 as well as
FIGURE 1. High expression of MARK2 in OC cells. (A) TCGA database showed the TRM of MARK2 in 426 human ovarian cancer as well as 88 normal tissues. (B) qPCR assays showed the mRNA levels of MARK2 in normal ovarian cell line and OC cell lines. (C) Immunoblot showed the protein levels of MARK2 in normal ovarian cell line and OC cell lines. ***: \( p < 0.001 \). MARK2: Microtubule affinity regulated kinase 2. IOSE80: Human normal ovarian epithelial cells 80.

FIGURE 2. The depletion of MARK2 inhibited proliferation of OC cells. (A) Immunoblot assays showed MARK2 expression in SK-OV-3 as well as A2780 cells upon the transfection of shControl, shMARK2 #1 and shMARK2 #2 plasmids. (B) CCK-8 assays showed the proliferation of SK-OV-3 as well as A2780 cells upon the transfection of shControl, shMARK2 #1 and shMARK2 #2 plasmids, and the OD450 value was measured. (C) Colony formation assays showed the proliferation of SK-OV-3 as well as A2780 cells upon the transfection of shControl, shMARK2 #1 and shMARK2 #2 plasmids, and the colony number was measured. **: \( p < 0.01 \); ***: \( p < 0.001 \). MARK2: Microtubule affinity regulated kinase 2. ShNC: sh-Negative Control.
A2780 cells, suggesting the promoting of apoptosis (Fig. 3B). Therefore, these results demonstrated that MARK2 induced the apoptosis of OC cells.

3.4 MARK2 ablation suppressed glucose metabolism of OC cells

MARK2 knockdown suppressed the glucose uptake in SK-OV-3 as well as A2780 cells (Fig. 4A). The Lactate production capacity in SK-OV-3 as well as A2780 cells was significantly decreased upon knockdown of MARK2 (Fig. 4B). Furthermore, we noticed that MARK2 knockdown decreased the expression of GLUT1, a marker of glucose metabolism, in SK-OV-3 as well as A2780 cells (Fig. 4C). Therefore, MARK2 ablation suppressed glucose metabolism of OC cells.

3.5 MARK2 regulated PI3K/AKT/p53 axis in OC

At last, we detected the mechanism underlying MARK2 promoting OC progression. Through Immunoblot assays, MARK2 knockdown decreased the phosphorylation of PI3K/AKT in SK-OV-3 as well as A2780 cells, suggesting the suppression of PI3K/AKT pathway (Fig. 5A). Additionally, the depletion of MARK2 suppressed the levels of p53 in SK-OV-3 and A2780 cells (Fig. 5B), further confirmed the regulation of MARK2 on the p53 pathway. Therefore, MARK2 regulated PI3K/AKT/p53 axis in OC.

4. Discussion

Ovarian cancer ranks first among the malignant tumors of female reproductive organs [14]. Despite improvements in surgical and chemotherapy techniques, relapse rates and drug resistance remain high [14]. Targeted therapy has become an effective way to treat ovarian cancer. Targeted therapy can enhance the lethality of tumor cells and reduce the damage to normal tissue, and is gradually applied to the clinical treatment of ovarian cancer, and shows advantages compared with traditional treatment [5, 14]. Targeted therapy options for ovarian cancer mainly include anti-angiogenic drugs, vaccines and anti-programmed cell death protein 1 (PD-1)/programmed cell death-ligand 1 (PD-L1) drugs [5, 14]. Once a patient has extensive metastasis, the disease progression can only be controlled as much as possible through targeted treatment, prolonging the survival period and improving the quality of life of patients, but it is difficult to achieve a cure [15]. To combat this disease, more targets still need to be developed [5]. Interestingly, here we found a member of the MARK family and a serine/threonine protein kinase, MARK2, was highly expressed in OC tissues and affected the progression of OC.

Through CCK-8 and colony formation assays, we found that MARK2 stimulated the growth of OC cells. By FCM and Enzyme-linked Immunosorbent Assay (ELISA) assays, we confirmed that MARK2 ablation induced apoptosis of ovarian cancer cells and inhibited glucose metabolism. These results suggested that MARK2 suppressed the development of OC in vitro. MARK2 is involved in the phosphorylation of proteins such as Tau, Cell Division Cycle Protein 25 (CDC25), and deacetylases such as Histone Deacetylase 7 (HDAC7) [7, 16]. In mammals, the MARK family consists of four members (MARK1–MARK4) [7, 16]. The role of MARK2 in tumors has been widely revealed. MARK2 enhanced the resistance of osteosarcoma cells to cisplatin [11]. MARK2 enhances aerobic glycolytic-mediated cell growth in breast cancer [12]. Similarly, we here also revealed its role in glucose metabolism in OC.

The PI3K/AKT pathway is involved in many biological processes, and its overactivation can lead to tumorogenesis [17, 18]. This pathway is closely related to OC, affecting OC cell proliferation, infiltration and chemotherapy resistance. Inhibitors developed against this signaling pathway, such as LY294002, have been shown to inhibit OC progression and serve as potential therapeutics [19, 20]. In addition, the positive rate of p53 in OC tissues is significantly higher than normal tissue and is positively correlated with histological grade, suggesting that abnormal expression of p53 plays an important role in the proliferation and development of tumor cells [21]. Studies on gastrointestinal tumors have found that tumors with high p53 mutation rate develop rapidly and are prone to metastasize [21]. Therefore, both the PI3K and p53 pathway could affect the OC progression. We here found that MARK2 mediated these two pathways, and the further mechanism needed to be further investigated.

5. Conclusions

In summary, we revealed that MARK2 was highly expressed in OC, and knockdown of MARK2 suppressed the proliferation and glucose metabolism of OC cells, as well as stimulated apoptosis of OC cells via PI3k/AKT/p53 axis.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

WJX, HYX, LH—designed the study and carried them out; WJX, HYX, YTW, YW—supervised the data collection, analyzed the data, interpreted the data, prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants or animals performed by any of the authors.

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Not applicable.
**FIGURE 3. Knockdown of MARK2 induced apoptosis of OC cells.** (A) FCM assays showed the apoptosis degree of SK-OV-3 as well as A2780 cells upon the transfection of shControl, shMARK2 #1 and shMARK2 #2 plasmids, and the percentage of apoptosis cells was measured. (B) Immunoblot assays showed cleaved caspase-3 as well as Bax expression in SK-OV-3 as well as A2780 cells upon the transfection of shControl, shMARK2 #1 and shMARK2 #2 plasmids. **: p < 0.01; ***: p < 0.001. MARK2: Microtubule affinity regulated kinase 2. shNC: sh-Negative Control. PI: Propyl iodide. FITC: Fluorescein isothiocyanate isomer I.
FIGURE 4. MARK2 ablation suppressed glucose metabolism of OC cells. (A) Glucose consumption in SK-OV-3 as well as A2780 cells upon the transfection of shControl, shMARK2 #1 and shMARK2 #2 plasmids were measured. (B) Lactate production in SK-OV-3 as well as A2780 cells upon the transfection of shControl, shMARK2 #1 and shMARK2 #2 plasmids were measured. (C) Immunoblot assays showed GLUT1 expression in SK-OV-3 as well as A2780 cells upon the transfection of shControl, shMARK2 #1 and shMARK2 #2 plasmids. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. MARK2: Microtubule affinity regulated kinase 2. sh-NC: sh-Negative control. GLUT1: Glucose transporter 1.
**FIGURE 5.** MARK2 regulated PI3K/AKT and p53 pathways in OC. (A) Immunoblot assays showed PI3K as well as AKT expression and phosphorylation in SK-OV-3 as well as A2780 cells upon the transfection of shControl, shMARK2 #1 and shMARK2 #2 plasmids. (B) Immunoblot assays showed p53 expression in SK-OV-3 as well as A2780 cells upon the transfection of shControl, shMARK2 #1 and shMARK2 #2 plasmids. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. MARK2: Microtubule affinity regulated kinase 2. sh-NC: sh-Negative control. PI3K: phosphatidylinositol 3-kinase. AKT: PKB (protein kinase B). p53: tumor suppressor protein 53.

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**CONFLICT OF INTEREST**
The authors declare no conflict of interest.

**REFERENCES**


Natarajan SR, Ponnusamy L, Manoharan R. MARK2/4 promotes Warburg effect and cell growth in non-small cell lung carcinoma through the AMPKα1/mTOR/HIF-1α signaling pathway. BBA Molecular Cell Research. 2022; 1869: 119242.


