SIN-CHN SCIENTIFIC PRESS PTE.LTD.

Molecular & Cellular Biomechanics 2025, 22(4), 1390.
https://doi.org/10.62617/mcb1390

Article

The effect and mechanism of AKT protein kinase activation on the biological behavior of Caki-2 cells

Shiqi Ye[1,*,†], Yin Zheng[2,†]

[1] School of Medicine and Population Health, The University of Sheffield, Sheffield S10 2TN, United Kingdom
[2] School of Mathematical and Physical Sciences, The University of Sheffield, Sheffield S10 2TN, United Kingdom
*** Corresponding author:** Shiqi Ye, qiye2333@yeah.net
[†] These authors contributed to the work equally and should be regarded as co-first authors.

CITATION

Ye S, Zheng Y. The effect and mechanism of AKT protein kinase activation on the biological behavior of Caki-2 cells. Molecular & Cellular Biomechanics. 2025; 22(4): 1390. https://doi.org/10.62617/mcb1390

ARTICLE INFO

Received: 17 January 2025
Accepted: 28 February 2025
Available online: 7 March 2025

COPYRIGHT

Copyright © 2025 by author(s). *Molecular & Cellular Biomechanics* is published by Sin-Chn Scientific Press Pte. Ltd. This work is licensed under the Creative Commons Attribution (CC BY) license. https://creativecommons.org/licenses/by/4.0/

Abstract: Objective: To investigate the effects and mechanisms of Protein Kinase B (AKT) activation on the biological behavior of malignant B-cells. **Methods:** This study used immunohistochemical staining to detect the differences between AKT tumor tissue and short and fat tissue. The RCC cell line Caki-2 was screened using real-time fluorescence quantitative PCR detection and Western blot assay. Analyze the effect of AKT activation on cell adhesion and migration through biomechanical experiments.in order to select the best siRNA experimental group. The effects of CCK8, the Transwell invasion experiment, and flow cytometry on Caki cell proliferation, invasion, migration, apoptosis, and cell cycle were detected. **Results:** According to the immunohistochemical staining results, it was found that compared to the surrounding normal tissues, AKT1 was expressed higher in tumor tissues ($P < 0.05$). After AKT activation, it can affect cell adhesion and regulate the function of integrins by phosphorylating various substrates. Integrins are cell adhesion molecules that can mediate cell adhesion to the extracellular matrix. AKT can enhance the adhesion between cells and matrix by phosphorylating integrins or their related signaling molecules. After AKT activation, it can promote the migration and invasion ability of cells. This is mainly achieved by activating multiple signaling pathways, such as Rac and Rho family proteins. These signaling pathways play important roles in cytoskeleton remodeling and cell movement. Phosphorylated AKT can activate these pathways, thereby promoting cell migration. AKT activation in Caki-2 cells via siRNA transfection demonstrated that AKT activation promoted the proliferation, invasion, After Caki-2 cell migration, AKT can transition Caki-2 cells from the G1 phase to the *S* phase. **Conclusion:** AKT may be involved in the malignant growth of RCC and holds potential as a therapeutic target.

Keywords: AKT; activation; clear cell renal carcinoma (CCRC); biological behavior

1. Introduction

Human renal clear cell carcinoma cells (Caki-2), which exhibit epithelial-like morphology and adherent growth characteristics, were initially discovered in the tissue of a primary renal adenocarcinoma from a 69-year-old Caucasian male [1]. Currently, they have extensive application value in drug screening and can be used for studying the pathogenesis of renal cancer. Can clarify the pathogenesis of kidney cancer. Renal cell carcinoma is also known as renal cell carcinoma. Its clinical manifestation is that epithelial cells have malignant tumors [2], which is a common tumor in the urinary system. It has a high incidence rate. Currently, surgical treatment is the main treatment for kidney cancer, but there are still some patients who experience lesion metastasis and recurrence after surgery [3]. In addition, kidney cancer has low sensitivity to

traditional chemotherapy and radiotherapy, and its pathogenesis is not clear enough [4], necessitating further exploration of novel therapeutic targets as treatment strategies to improve patients' quality of life [5]. Revealing the key role of the PI3K/Akt/mTOR pathway in Caki-2 cells, PI3K serves as an upstream signaling molecule that activates Akt to regulate mTOR activity, forming an important signaling pathway. Analyzing the comprehensive regulatory effects of this pathway on Caki-2 cell proliferation, metabolism, apoptosis, and autophagy, based on a deep understanding of the mechanism of this pathway, drug intervention strategies targeting key nodes (such as PI3K, Akt, mTOR, etc.) are proposed to inhibit Caki-2 cell proliferation and induce apoptosis, providing a theoretical basis for clinical applications.

AKT can also be referred to as protein kinase B, becoming activated upon binding of its pleckstrin homology domain to P13K, subsequently mediating roles in cell apoptosis and proliferation [6]. AKT activation is a highly complex process involving numerous signaling pathways. Cells, upon receiving external signals, undergo a series of molecular reactions leading to AKT activation [7]. During phosphorylation, AKT can be activated by kinase phosphorylation, PDK1 is phosphorylated and located at the Thr308 site, and mTORC2 is a target phosphorylation in mammals, while Mammalian Target of Rapamycin Complex 2 (mTORC2) phosphorylates the Ser473 site [8]. The phosphorylation of these two sites is crucial for AKT activation. In renal cancer cells, AKT protein kinase activation is primarily caused by abnormalities in upstream signaling pathways. The P13K/AKT/mTOR signaling pathway is activated in renal cancer cells through various mechanisms, including growth factor receptor activation. The activation of AKT protein kinase plays a significant role in the biological behavior of renal cancer [9]. It affects renal cancer cell proliferation, apoptosis, angiogenesis, migration, and invasion through multiple mechanisms.

Based on this background, the present study aimed to investigate the effects and mechanisms of AKT protein kinase activation on the biological behavior of Caki-2 cells using immunohistochemical staining, real-time fluorescent quantitative PCR, Western blot analysis, CCK8 assay, Transwell invasion assay, wound healing assay, and flow cytometry. The specific report is as follows.

2. Materials and methods

2.1. Cell culture

Caki-2 is from the Chinese Academy of Sciences. Fetal bovine serum McCoy 5A culture medium is directly added into the culture bottle, humidified, and cultured in the incubator at 37 °C. The carbon dioxide concentration is 5%, and the cell density is 80%. After that, trypsin with a concentration of 0.25 is added for cell digestion.

2.2. Reagents

The required reagents for the experiment include AKT and pAKT antibodies, Western blot luminescence detection reagents, all purchased from Cell Signaling Company in the United States; The 3-phosphoglyceraldehyde dehydrogenase antibody was purchased from Santa Cruz Corporation in the United States; CelLytic M cell lysate is sourced from Sigma Corporation in the United States; BrdU primary antibody

is provided by the American company Chemicon; the protein quantification kit was purchased from Pierce Corporation in the United States.

2.3. Methods

2.3.1. Immunohistochemical staining

Clinical pathological specimens were obtained, embedded in paraffin, sectioned, and stained immunohistochemically. The microscope was inverted for observation. Renal cancer Caki-2 cells were placed on slides, fixed with formaldehyde using cytological preparations, and incubated at 4 ℃. After cell fixation, permeabilization was performed using 1% Triton X-100, followed by the addition of 3% H_2O_2. Goat serum was used for blocking, and Caki-2 primary antibody was added. The cell nuclei were stained with DAPI, and the slides were mounted with an antifluorescent quenching agent. Observations were made using a confocal microscope.

2.3.2. Real-time fluorescent quantitative PCR

Using a total RNA extraction kit, rigorously extract total RNA from cells and follow the instructions of the cDNA first strand synthesis kit to accurately perform the reverse transcription step. The forward primer was 5’-ATCCTACCTATCTGTACCGAC-3’, and the reverse primer was 5’-GGGTGAATTATTCTGACCTCC-3’. *β*-Actin was used as a reference gene with the forward primer 5’-CTTAGTTGCGTTACACCCTTTCTTG-3’ and the reverse primer 5’-CTGTCACCTTCACCGTTCCAGTTT-3’. Quantitative analysis was conducted using the Exicycler^{TM} 96 Fluorescent Quantitative PCR Instrument (BIONEER).

2.3.3. Western blot experiment

Add protein lysate to the sample to extract total protein, then place the mixture on ice for 40 min to promote protein release. Afterwards, take the supernatant and determine the protein content by the BCA method. Select 40 micrograms of protein sample and perform SDS-PAGE electrophoresis separation on the gel containing 10% polyacrylamide. After electrophoresis, transfer the protein onto a PVDF membrane at a voltage of 100 volts. Next, immerse the PVDF membrane in a sealing solution and seal it at 37 degrees Celsius for 1 h. After closure, continue to shake and incubate at 37 degrees Celsius for 1 hour, then wash the membrane surface with PBS solution. Afterwards, alkaline phosphatase-labeled secondary antibodies were added for incubation, and the grayscale values of the bands were quantitatively analyzed using ImageJ software.

2.3.4. Cell proliferation assay

Cells were seeded in a culture dish with a density of 300, and the culture medium was replaced every three days. Adjust the cell density appropriately, and then inoculate it into a 96-well plate. After the cells reach the appropriate density, add 100 microliters of MTT solution to each well and culture at 37 degrees Celsius for 4 h. Subsequently, remove the supernatant and add 200 microliters of dimethyl sulfoxide (DMSO) to each well. By measuring the absorbance (OD value) of each well, we can collect relevant data and further conduct data analysis.

2.3.5. Cloning assay

Cells were seeded and cultured at a density of 300 per dish, and the culture

medium was changed every 3 days. The entire cultivation process is carried out at 37 °C. The cells were fixed after washing with PBS, and then the formaldehyde fixative was removed. Next, wash the cells three times with PBS washing solution, and then stain the cells with a composite dye for 10 min. After staining, rinse with clean water three times and finally count the cells.

2.3.6. Flow cytometry analysis

To detect the cell cycle, the cells were first digested with trypsin, then fixed with a 70% pre-cooled solution, and treated overnight. Next, propidium iodide and RNase were added to the cells and incubated at 37 °C for 30 min. Afterwards, the percentage of different cell cycles was measured using flow cytometry. For the detection of cell apoptosis, when the cell density reaches 80%, trypsin digestion is used, and the cells are washed with PBS. Then, resuspend the cells using Annexin V from the apoptosis assay kit and buffer, and add 5 microliters of FITC-labeled Annexin V solution. After incubating at room temperature in the dark for 15 min, detection was performed using a flow cytometer.

2.3.7. Biomechanical experiments

Inoculate Caki2 cells directly into a culture dish and cultivate them to the desired density using complete culture medium. During the cultivation process, set the temperature to 37 °C and the gas environment to 95% air + 5% carbon dioxide. Complete cell counting through trypsin digestion to ensure experimental consistency.

2.4. Statistical methods

During data analysis, statistical software SPSS 24.00 was used to complete the analysis. Quantitative data were presented as mean ± standard using a *t*-test, while technical data were presented as frequency and frequency using a chi-square test. When $P < 0.05$, statistical significance was indicated.

3. Results

3.1. Differential expression of AKT in tumor tissues and adjacent non-tumor tissues

The results of this study demonstrate that the expression of AKT1 in tumor tissues is significantly higher than that in adjacent normal tissues, with statistical significance in the data comparison ($P < 0.05$). This is specifically shown in **Figure 1**.

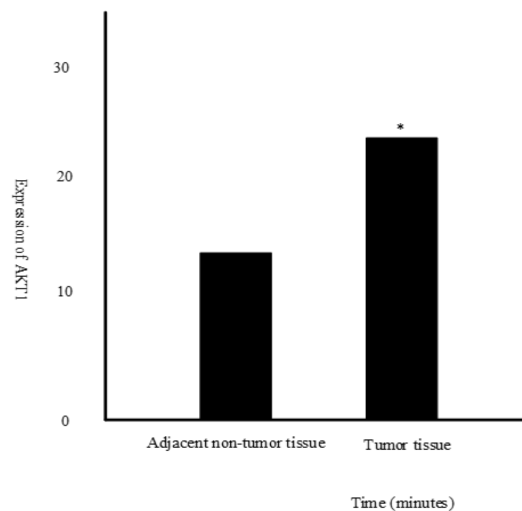

Figure 1. Differential expression of AKT in tumor tissues and adjacent non-tumor tissues.

Note: "*" indicates that compared to the control group, it has statistical significance ($P < 0.05$); the same applies below.

3.2. Proliferation and cloning of Caki-2 cells

After activating Caki-2 cells with siRNA targeting AKT, it was found that AKT effectively inhibited the proliferation of Caki-2 cells, with a significantly lower proliferation rate compared to the control group. This difference was maintained and became more pronounced at 96 h and 120 h post-activation ($P < 0.05$). The specific results are shown in **Figure 2**.

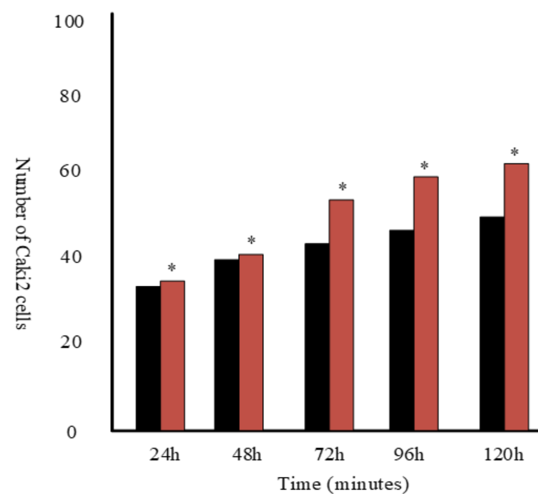

Figure 2. Analysis of Caki-2 cell proliferation results.

3.3. Activation of AKT protein kinase can induce cell cycle arrest

The experimental results showed that compared with the control group, the number of cells in the G0/G1 phase significantly increased after shRNA transfection. At the same time, the proportion of *S* phase cells decreased accordingly. This indicates that the activation of AKT can accelerate the transition of Caki-2 cells from the G1 phase to the *S* phase.

3.4. Activation of AKT protein kinase promotes apoptosis in Caki-2 cells

The effect of AKT protein kinase activation on cell apoptosis was analyzed in depth using cell staining techniques and flow cytometry. The research results showed that the characteristics of apoptotic bodies were significant in the transfected cells. Compared with the control group, the proportion of cell apoptosis after AKT protein kinase activation was significantly higher in the siRNA transfection negative control group and untreated blank control group. In addition, Western blot experiments further revealed that Caki2 cells can lead to a decrease in Bcl-2 protein expression levels and an increase in Caspase-3 protein expression after transfection. These findings collectively indicate that the activation of AKT protein kinase has the ability to induce cell apoptosis, as shown in **Figure 3**.

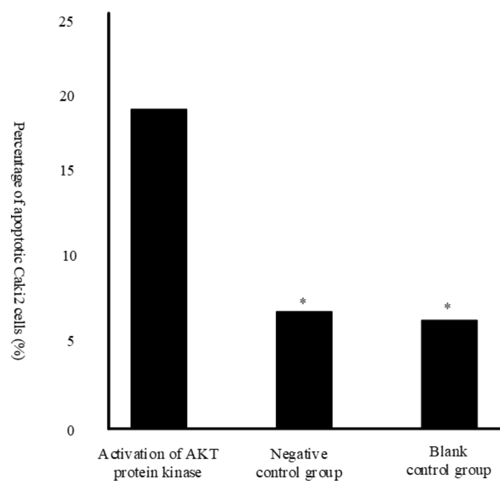

Figure 3. Effect of AKT protein kinase activation on apoptosis in Caki-2 cells.

3.5. Results of biomechanical experiments

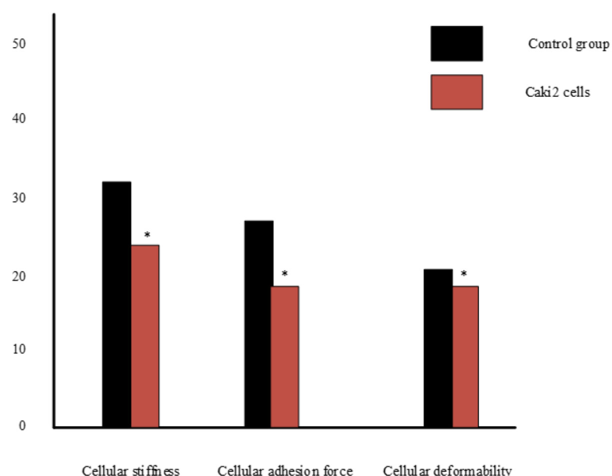

Figure 4. Analysis of biomechanical experiment results.

Stiffness testing was conducted using AFM equipment, revealing that Caki-2 cells exhibited a relatively high stiffness value, indicating their high rigidity. Compared to other cell types, the stiffness value of Caki-2 cells was significantly higher. In the analysis of cell adhesion results, Caki-2 cells were found to have good

attachment ability compared to the control group. During the cell deformability test, it was observed that Caki-2 cells had a certain resistance under mechanical stimulation, with limited deformability. These findings are specifically shown in **Figure 4**.

4. Discussion

RCC, as a very common malignant tumor in the human body. An epidemiological study has clearly stated [10] that over 100,000 people lose their lives to RCC each year. Currently, surgical treatment is the primary means of managing RCC; however, approximately 25% of patients still experience recurrence and metastasis after surgery. RCC exhibits low sensitivity to conventional chemotherapy, and there are no targeted treatment options for advanced RCC [11–14]. The pathogenesis of RCC is very complex, including genetic changes and activation of signaling pathways. AKT can be an important component of the P13K/AKT/mTOR signaling pathway, and such abnormal activation may promote cell proliferation and apoptosis, inhibit apoptosis, and enhance invasive and migratory abilities. Therefore, exploring novel therapeutic targets and developing effective treatment strategies for the pathogenesis of RCC is crucial to improving patients' quality of life [15]. Cell proliferation, apoptosis, and the cell cycle all impact the biological functions of cells. AKT is an important signaling pathway in intracellular signal transduction, and its abnormal activation may be closely related to tumorigenesis. AKT protein kinase, a serine-threonine kinase, plays a vital role in cell signaling, particularly in insulin signaling and the regulation of cell survival [16]. Structurally, AKT contains an N-terminal functional domain, a catalytic functional domain, and hydrophobic motifs. AKT activation typically involves After phosphorylation of Thr308, growth factor-mediated AKT is located at the Thr308 site, which is also one of the key activation steps of AKT [17], while phosphorylation at the Ser473 site is considered necessary for full activation of AKT protein kinase function. Caki-2 cells, derived from human papillary RCC, are significant in cancer research [18–20]. Although direct studies on AKT protein kinase and Caki-2 cells may be scarce, the importance of AKT in tumor cell growth and survival [21] suggests its relevance. Upon AKT activation, downstream target proteins such as p21, p27, and FOXOs in Caki-2 cells [22] regulate the cell cycle and cell proliferation, affecting the proliferative capacity of Caki-2 cells. AKT also regulates Caki-2 cells by modulating glucose, lipid, and protein metabolism [23–25], such as through mTORC1, 4E-BP1, and S6k, further influencing Caki-2 cell metabolism. Biomechanics research focuses on the behavioral changes of cells in a mechanical environment, and the activation state of AKT is closely related to the mechanical properties of cells. In Caki-2 cells, activation of AKT may lead to remodeling of the cytoskeleton and changes in cell adhesion, thereby affecting cell migration, invasion, and mechanical sensitivity. AKT activation leads to the remodeling of the cytoskeleton by phosphorylating actin-binding proteins and regulating actin polymerization. This restructuring may enhance the rigidity and stability of cells, which is beneficial for cell migration and invasion. Meanwhile, the reconstruction of the cytoskeleton may also affect the mechanical sensitivity of cells, enabling them to better adapt to changes in the mechanical environment.

The activation of AKT is a multi-step cascade reaction that is regulated by various

intracellular and extracellular stimuli, including growth factors, hormones, and biomechanical factors. When cells are stimulated by external factors, these stimuli will ultimately act on AKT through a series of signaling pathways, activating it. Biomechanical factors, such as tensile, compressive, or shear forces on cells, can indirectly regulate the activation of AKT by affecting the cytoskeleton, cell membrane, and signaling molecules within the cell. Cell proliferation is a strictly regulated biological process that involves various stages of the cell cycle and the synergistic effects of multiple signaling pathways. The biomechanical properties play an important role in cell proliferation. On the one hand, the mechanical stimulation received by cells can affect the progression of the cell cycle, thereby regulating the rate of cell proliferation. For example, appropriate compressive stress can promote the proliferation and differentiation of osteoblasts, while excessive compressive stress can lead to cell apoptosis. On the other hand, the rearrangement of the cytoskeleton and changes in cell morphology can also affect the proliferation ability of cells. For example, during the EMT process, the cytoskeleton of melanoma cells undergoes depolymerization, resulting in a decrease in cell stiffness, which enhances the migration and invasion ability of cells and may also affect the proliferation rate of cells.

The activation of AKT is a multi-step cascade reaction. When cells are stimulated by growth factors, hormones, or other stimuli, phosphoinositol 3-kinase (PI3K) is activated, catalyzing the conversion of phosphatidylinositol 4,5-diphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 subsequently binds to the N-terminal PH domain of AKT, recruiting AKT onto the cell membrane. On the cell membrane, AKT is phosphorylated and activated by phosphatidylinositol-dependent kinase 1 (PDK1) and mammalian rapamycin target protein complex 2 (mTORC2), phosphorylating their Thr308 and Ser473 sites, respectively.

Caki-2 cells are a type of human renal clear cell carcinoma derived from early-stage renal adenocarcinoma tissue of a 69-year-old white male. This type of cell has specific growth and proliferation characteristics under in vitro culture conditions and is an important tool for studying the biological behavior and treatment strategies of renal cancer. The activation of AKT activates the mTOR pathway through phosphorylation, promoting protein synthesis and cell growth, thereby accelerating the proliferation of Caki-2 cells. This is of great significance for the development and progression of kidney cancer. AKT promotes the survival of Caki-2 cells by inhibiting pro-apoptotic proteins such as Bad and Caspase-9 and activating anti-apoptotic proteins such as Bcl-2. This helps renal cancer cells resist apoptotic signals, thereby prolonging their survival time. AKT participates in the regulation of glucose metabolism, lipid metabolism, and protein metabolism, affecting the energy metabolism of Caki-2 cells. This regulatory effect helps renal cancer cells adapt to different microenvironments and meet their energy requirements for rapid proliferation. AKT promotes the proliferation and migration of endothelial cells, thereby promoting angiogenesis. This is crucial for tumor angiogenesis and blood supply in renal cancer.

As a key member of the PI3K/Akt signaling pathway, AKT activation is regulated by the upstream regulatory factor PI3K. When PI3K is activated, it catalyzes the conversion of PIP2 to PIP3, which then recruits and activates AKT. Activated AKT

subsequently regulates the activity of downstream target proteins such as mTOR and GSK3 β through phosphorylation, thereby participating in the regulation of biological processes such as cell proliferation, survival, and metabolism. Activate or inhibit downstream substrates such as apoptosis-related proteins and metabolism-related enzymes through phosphorylation. The phosphorylation state changes of these substrates can affect their activity or stability, thereby regulating the biological behavior of Caki-2 cells. The AKT signaling pathway interacts with other signaling pathways such as the NF-κB pathway, MAPK pathway, Wnt pathway, etc. These interactions will affect the activity of the AKT signaling pathway and the regulation of downstream target proteins, thereby further regulating the biological behavior of Caki-2 cells.

AKT protein kinase, as a key molecule in the PI3K/Akt signaling pathway, plays an important role in regulating cell proliferation, survival, metabolism, and migration. These changes in biological behavior may indirectly affect the biomechanical properties of cells, such as cell stiffness, adhesion, migration ability, etc. The activation of AKT may affect the stiffness of cells by regulating the rearrangement of the cytoskeleton and the interaction of the extracellular matrix. The cytoskeleton is an important structure for maintaining cell morphology and stiffness, and the activation of AKT can affect the dynamic balance of cytoskeletal proteins such as actin and microtubules. In addition, AKT may indirectly affect the stiffness of cells by regulating the synthesis and degradation of extracellular matrix components such as collagen and fibronectin. By regulating the expression and activity of cell adhesion molecules, it affects the adhesion between cells and the extracellular matrix. Cell adhesion molecules are important molecules that mediate interactions between cells and between cells and the matrix. Their expression and activity are regulated by multiple signaling pathways, including the PI3K/Akt pathway. Changes in adhesion may affect biological behaviors such as cell migration, invasion, and colony formation. Moreover, AKT activation plays an important role in cell migration. It can promote or inhibit cell migration by regulating the rearrangement of the cytoskeleton, the establishment of cell polarity, and the interaction between cells and the matrix. In Caki-2 cells, the activation of AKT may affect cell migration ability through similar mechanisms, thereby having an important impact on tumor invasion and metastasis. However, it should be noted that although the potential impact of AKT on the biomechanical properties of Caki-2 cells can be inferred from its general role in cellular biology, these speculations still require experimental evidence to support. At present, there is still limited research on the specific effects and mechanisms of AKT protein kinase activation on the biomechanical properties of Caki-2 cells, and more experiments are needed in the future to prove it.

AKT activation can alter the characteristics of Caki-2 cells, including cell proliferation, apoptosis, migration, and cell cycle. AKT activation can promote the proliferation of Caki-2 cells. In cell signaling, AKT, as a key molecule in the PI3K/Akt signaling pathway, can be activated to transmit mitotic signals, upregulate the expression of cell cycle proteins (such as cyclinD1), promote the transition of cells from G1 phase to S phase, and accelerate the progression of the cell cycle. This process is crucial for cell proliferation. AKT activation can inhibit apoptosis of Caki-2 cells. AKT regulates the process of cell apoptosis by phosphorylating a series of substrate

proteins, such as Bad and GSK-3β. Among them, Bad is a pre-apoptotic member of the Bcl-2 family, and AKT phosphorylation of Bad can block its interaction with Bcl-2 or Bcl-XL, thereby inhibiting the anti-apoptotic activity of these two molecules. In addition, AKT can regulate the process of cell apoptosis by phosphorylating other apoptosis-related molecules. AKT activation can enhance the migration and invasion ability of Caki-2 cells. This is mainly achieved by regulating the rearrangement of the cytoskeleton, the establishment of cell polarity, and the interaction between cells and the matrix. AKT activation can promote the formation and rearrangement of actin fibers, thereby enhancing the migration ability of cells. At the same time, AKT can also regulate the synthesis and degradation of extracellular matrix components such as collagen and fibronectin, as well as affect the expression and activity of cell adhesion molecules such as integrins, to regulate the interaction between cells and the matrix, thereby enhancing the invasive ability of cells. In addition to promoting the transition of cells from G1 phase to *S* phase as mentioned above, AKT activation can also affect the cell cycle through other mechanisms. For example, AKT can inhibit the expression of fork-related transcription factors mediated by p27, a cyclin-dependent kinase inhibitor whose downregulation can promote cell cycle progression. In addition, AKT can regulate the cell cycle by affecting the activity of other cell cycle-related proteins such as CDK4, CDK2, etc. AKT activation can promote the expression and activity of certain adhesion molecules (such as integrins, cadherins, etc.), thereby enhancing the adhesion between cells or between cells and matrix. AKT also plays an important role in establishing cell polarity. The formation of cell polarity helps cells adhere to other cells or matrix in a specific direction, thereby further affecting cell adhesion. Promote the synthesis and rearrangement of cytoskeletal proteins such as actin and microtubules, thereby enhancing the skeletal structure of cells and increasing their stiffness. By regulating the synthesis and degradation of extracellular matrix components such as collagen and fibronectin, as well as by affecting the expression and activity of cell adhesion molecules such as integrins, the interaction between cells and the matrix is regulated. These changes may also affect the stiffness of cells.

In this study, it was found that compared to adjacent tissues, AKT1 expression was higher in the tumor group after immunohistochemical staining. ($P < 0.05$), suggesting that AKT1 expression is elevated in tumor tissues. After transfection with siRNA, Caki-2 cells were effectively activated, promoting cell proliferation and inhibiting cell invasion. Activation of AKT protein kinase exerts a blocking effect on the cell cycle. Through shRNA transfection, the number of cells in the G0/G1 phase increased to a certain extent, while the proportion of cells in the S phase decreased. AKT activation effectively promoted the transition of Caki-2 cells from the G1 phase to the S phase. In the analysis of the correlation between AKT protein kinase activation and Caki-2 cell apoptosis, cell staining and flow cytometry were used to assess the impact of AKT protein kinase activation on apoptosis. The study results directly show that transfected cells exhibited apparent apoptotic bodies, and the proportion of apoptotic cells with AKT protein kinase activation was significantly higher than that in the siRNA transfection negative control group and the blank control group. Additionally, through Western blot experiments, Caki-2 cell transfection reduced Bcl-2 protein expression to a certain extent and increased Caspase-3 protein expression, suggesting that AKT protein kinase activation can induce cell apoptosis.

In summary, AKT protein kinase activation plays a certain role in the cell biology of Caki-2 cells. The high expression of AKT1 in tumor tissues compared to adjacent non-cancerous tissues, its promotion of Caki-2 cell proliferation, inhibition of cell invasion and apoptosis, increase in the number of cells in the G0/G1 phase, and induction of cell apoptosis may be closely related to the malignant growth of renal cell carcinoma, making it a potential therapeutic target.

Author contributions: Conceptualization, SY and YZ; methodology, SY; software, YZ; validation, YZ; formal analysis, SY; investigation, YZ; resources, YZ; data curation, YZ; writing—original draft preparation, SY; writing—review and editing, SY; visualization, SY and YZ; supervision, SY and YZ; project administration, SY and YZ; funding acquisition, SY. All authors have read and agreed to the published version of the manuscript.

Ethical approval: Not applicable.

Conflict of interest: The authors declare no conflict of interest.

References

1. Asnaghi R, Antonarelli G, Battaiotto E, et al. An update on promising and emerging protein kinase B/AKT inhibitors for breast cancer. Expert Opinion on Pharmacotherapy. 2025; 26(3): 235-247. doi: 10.1080/14656566.2025.2454290
2. Qin H, Liu W, Jing Y, et al. Clinical significance of DTX2 expression in clear cell renal cell carcinoma tissues based on TCGA database analysis and its impact on renal cancer cell proliferation, migration, and invasion. Chinese Journal of Cancer Biotherapy. 2024.
3. Pang Y, Wei J, Shang Q, et al. 68Ga-Fibroblast Activation Protein Inhibitor, a Promising Radiopharmaceutical in PET/CT to Detect the Primary and Metastatic Lesions of Chromophobe Renal Cell Carcinoma. Clinical Nuclear Medicine. 2020; 46(2): 177-179. doi: 10.1097/rlu.0000000000003454
4. Wang H, Zhu W, Xie C, et al. The impact of Josephin domain-containing protein 2 on the proliferation and migration abilities of clear cell renal cell carcinoma. Chinese Journal of Experimental Surgery. 2024.
5. Cai T, Zhu Z, Xu C, et al. Expression and significance of fibroblast growth factor receptor 2 in clear cell renal cell carcinoma. Journal of Peking University (Health Sciences). 2022.
6. Rao H, Li X, Liu M, et al. Di-Ras2 promotes renal cell carcinoma formation by activating the mitogen-activated protein kinase pathway in the absence of von Hippel–Lindau protein. Oncogene. 2020; 39(19): 3853-3866. doi: 10.1038/s41388-020-1247-y
7. Shamsuri AS, Sim EUH. In silico prediction of the action of bromelain on PI3K/Akt signalling pathway to arrest nasopharyngeal cancer oncogenesis by targeting phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha protein. BMC Research Notes. 2024; 17(1). doi: 10.1186/s13104-024-06995-2
8. Ding Z, Zhou X, Ma L. Expression and function of miR-199b-5p in type 2 papillary renal cell carcinoma with inferior vena cava tumor thrombus. Journal of China-Japan Friendship Hospital. 2021.
9. Tyagi K, Roy A, Mandal S. Protein kinase C iota promotes glycolysis via PI3K/AKT/mTOR signalling in high grade serous ovarian cancer. Molecular Biology Reports. 2024; 51(1). doi: 10.1007/s11033-024-09918-3
10. Chen Z, Yang Z, Wang X. The impact of lysyl oxidase-like 2 on the proliferation and migration of clear cell renal cell carcinoma. Chinese Journal of Experimental Surgery. 2022.
11. Feng H, He H. The impact of exosome AP000439.2 derived from clear cell renal cell carcinoma on M2 macrophage polarization. International Journal of Urology and Nephrology. 2024.
12. Mo C, Dai Y, Kang N, et al. Ectopic Expression of Human MutS Homologue 2 on Renal Carcinoma Cells Is Induced by Oxidative Stress with Interleukin-18 Promotion via p38 Mitogen-activated Protein Kinase (MAPK) and c-Jun N-terminal Kinase (JNK) Signaling Pathways. Journal of Biological Chemistry. 2012; 287(23): 19242-19254. doi: 10.1074/jbc.m112.349936

13. Peng H, Wu Y, Li J, et al. Long non-coding RNA MALAT-1 regulates proliferation, apoptosis, and invasion of renal cancer cells through the EZH2-β-catenin signaling pathway. Journal of Modern Urology. 2020.
14. Hu B, Liu S, Huang Y, et al. LncRNA RP13-991F5.2 inhibits the invasion and proliferation of renal cell carcinoma cells by regulating the LKB1/AMPK signaling pathway. Journal of Shanxi Medical University. 2020.
15. Thaklaewphan P, Wikan N, Potikanond S, et al. Oxyresveratrol Enhances the Anti-Cancer Effect of Cisplatin against Epithelial Ovarian Cancer Cells through Suppressing the Activation of Protein Kinase B (AKT). Biomolecules. 2024; 14(9): 1140. doi: 10.3390/biom14091140
16. Seo JH, Rah JC, Choi SH, et al. RETRACTION: α-Synuclein regulates neuronal survival via Bcl-2 family expression and PI3/Akt kinase pathway. The FASEB Journal. 2024; 38(16). doi: 10.1096/fsb2.70007
17. Zhang W, Hu Z, Fu Q, et al. miR-124 affects the malignant biological behavior of OS-RC-2 cells in renal cell carcinoma by regulating the Jagged1/Notch signaling pathway. Chinese Journal of Cancer Biotherapy. 2022.
18. Wang Q, Huang X, Hu Y, et al. Mechanism of TCTP regulating proliferation and migration of renal cell carcinoma cells. Journal of Naval Medical University. 2024.
19. Bögel G, Sváb G, Murányi J, et al. The role of PI3K-Akt-mTOR axis in Warburg effect and its modification by specific protein kinase inhibitors in human and rat inflammatory macrophages. International Immunopharmacology. 2024; 141: 112957. doi: 10.1016/j.intimp.2024.112957
20. Yang Z, Yang Y, Jiang Y, et al. Expression of collagen type IV alpha 4 in clear cell renal cell carcinoma and its correlation with prognosis and immune infiltration. Practical Oncology Journal. 2024.
21. Oya M, Mizuno R, Shinojima T, et al. 362: Inhibition of the ubiquitin-proteasome pathway activates mitogen-activated protein kinases and induces apoptosis in renal cancer cells. The Journal of Urology. 2005; 173(4S): 99-99. doi: 10.1016/S0022-5347(18)34615-9
22. Wang L, Huang G, Jiang W, et al. Methylation degree of miRNA-4729 in renal cancer tissues and its impact on the proliferation and migration abilities of renal cancer cells. Oncology Research and Clinical Treatment; 2024.
23. Shen X, Li Q, Wang G, et al. LncRNA ARHGAP5-AS1 inhibits renal cancer cell proliferation and invasion by targeting miR-155-5p. International Journal of Surgery. 2024.
24. Cianfrocca R, Rosanò L, Spinella F, et al. Retraction: β-arrestin-1 mediates the endothelin-1-induced activation of Akt and integrin-linked kinase. Canadian journal of physiology and pharmacology. 2024.
25. Wang X, Hu W, Meng L. LINC00462 affects glycolysis in clear cell renal cell carcinoma cells through the MYC/ABCC3 axis and regulates their sensitivity to cisplatin. Chinese Journal of Cancer Biotherapy. 2023.