Gαi1 Promoted Proliferation, Migration and Invasion via Activating the Akt-mTOR/Erk-MAPK Signaling Pathway in Renal Cell Carcinoma

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Background: Renal cell carcinoma (RCC) accounts for about 2–3% of all adult malignancies. G protein alpha inhibitory subunit 1 (Gαi1) plays a key role in mediating PI3K-Akt signaling upon activation of receptor tyrosine kinases (RTKs). However, little is known about its expression, regulation and biological function in RCC.

Methods: Gαi1 expression in RCC tissues and cells was detected by quantitative real-time PCR (qRT-PCR), Western blot and immunohistochemistry (IHC). The effect of Gαi1 silence on cell proliferation and apoptosis of 786-O and ACHN cells was detected by CCK-8 assay and flow cytometry. Wound-healing assay and Transwell assays were used to detect the cell invasion in RCC cells. The expression of CDK4, cyclin D1, MMP-2, MMP-9, Bax, Bcl-2, p/t-Akt, p/t-S6 and p/t-Erk was detected by Western blot and qRT-PCR. Furthermore, a nude mouse subcutaneous xenograft model was used to further evaluate the potential effects of Gαi1 in vivo.

Results: In the present study, our data showed that Gαi1 expression was dramatically increased in RCC tissues compared with normal renal tissues. In addition, knocking down the expression of Gαi1 subsequently inhibited proliferation, migration and invasion of RCC cells in vivo and vitro. Furthermore, the expression of CDK4, cyclin D1, MMP-2 and MMP-9 was significantly reduced upon Gαi1 inhibition. Gαi1 positively regulates the activation of the mTOR and Erk pathways.

Conclusion: In conclusion, this study reveals Gαi1 promoted proliferation via activating the Akt-mTOR and Erk-MAPK signaling pathways in RCC, and Gαi1 may be a therapeutic and prognostic target for RCC.

Keywords: renal cell carcinoma, Gαi1, cell proliferation, cell migration, therapeutic targets

Introduction
Renal cell carcinoma (RCC) was the most common type of malignant tumor in kidney that accounts for more than 90% of renal cancer,1,2 among which 75–85% of them are clear cell renal cell carcinoma.3 RCC ranks among the 10 most common cancer types globally that causes about 140,000 deaths every year.4 Despite the continuous progress in medical treatment, the incidence of the disease has increased year by year.5 Patients with localized renal tumors generally have a favorable prognosis, with 5-year OS rates consistently between 60% and 70% after surgery.6 However, due to RCC is not sensitive to chemotherapy, the treatment options are very limited.7 The standard treatment for RCC is surgical resection.8 The prognosis of these patients with advanced disease is extremely poor, and the
5-year overall survival (OS) rate for patients with metastatic disease is less than 10%.8,9 Although immunotherapy, including interleukin-2 and interferon-α, is widely used in the treatment of metastatic renal cell carcinoma (mRCC), its efficacy is still controversial.10–12 Targeted drugs, including vascular endothelial growth factor receptor (VEGFR) inhibitors and rapamycin (mTOR) pathway inhibitors, provide new treatment regimens for mRCC patients. But the effect of these treatments is very limited and some patients suffer serious side effects such as hand–foot skin reactions, hypertension, diarrhea, etc.13,14 Thus, it is essential to identify the molecular mechanism underlying RCC to provide guidance for clinical decision-making and developing novel therapeutic strategies.

The G protein α inhibitory subunit (including Ga1, Ga2, and Ga3) were initially identified by their ability to bind to GPCRs (G protein-coupled receptors) and inhibit adenylylate cyclase (AC) activity.15 The traditional view is that G protein α inhibitory subunit (Ga) routinely transduces G protein-coupled receptor (GPCR) signals, inhibits adenylylate cyclase (AC) activity, and reduces the level of intracellular cAMP.16,17 Recent studies have shown that Ga1/3 are physically bound to ligands that activated RTK (such as EGF and KGF), which encodes and activates the adaptor protein Gab1 to mediate downstream Akt-mTOR activation.18–20 Upon stimulation by EGF, Ga1 can bind epidermal growth factor receptor (EGFR) as an adaptor protein, and promote proliferation and migration through the PI3K-Akt-mTOR pathway in vitro.21,22 In addition, Ga1 mediates the activation of Akt-mTOR pathway induced by keratinocyte growth factor (KGF) and basic fibroblast growth factor (bFGF), and thus participates in the regulation of proliferation, differentiation, survival and migration of skin keratinocytes.19,20,22 In human gliomas, overexpression of Ga1 leads to Akt activation and proliferation of glioma cells.23 However, the role of Ga1 in the progression of RCC remains unclear.

This study aimed to investigate the expression and regulatory mechanisms of Ga1 in RCC. Our study indicates that Ga1 is highly expressed in RCC tissues. Our results further showed that suppression of Ga1 expression significantly inhibited proliferation of RCC cells in vitro and the tumor growth of RCC cells in nude mice. In addition, the decline of CDK4, cyclin D1, MMP-2 and MMP-9 was significant after inhibiting Ga1 in RCC cells. Furthermore, we found that the Akt-mTOR and Erk-MAPK pathways are downstream pathways of Ga1. Together, our data suggest that Ga1 may be a potential therapeutic target for RCC patients.

Materials and Methods
Clinical Samples
From 2017 to 2019, altogether 43 paired RCC and adjacent normal tissue samples were collected from patients who were undergoing renal cancer surgery in The Second Affiliated Hospital of Nantong University. The patients did not receive any treatment before surgery. All patients participating in the study were informed and written informed consent was obtained. The study was approved by The Second Affiliated Hospital of Nantong University Ethics Committee, according to the Declaration of Helsinki.

Cell Culture
Human renal cell carcinoma cell lines 786-O, A498, ACHN, Caki1 and Caki2 were purchased from the Shanghai Institutes of Biological Sciences (Shanghai, China). 786-O, Caki1 and Caki2 Cells were cultured in 1640 medium (Gibco) supplemented with 10% fetal bovine serum (Gibco) and A498, ACHN cells were maintained in MEM (Gibco) medium with 10% FBS in a humidified incubator (Thermo Fisher Scientific) air atmosphere containing 5% CO2.

Lentiviral Infection
Ga1 expression was knocked down using lentiviral vectors. The LV-shGa1 sequences were as follows: 5'-AGGATCAACACATATGAA-3' and LV-shNC sequences were as follows: 5'-TTCTCCCAGTCGGTACC-3'. Ga1 knockdown (named as LV-shGa1) lentivirus and negative control GV248 vector (termed as LV-shNC) infected the 786-O and ACHN cells, following the reagent manufacturer’s instructions (Genechem, Shanghai, China). Green fluorescence cells were observed with the fluorescence microscope and retrovirial production and puromycin stable cell selection has been previously described cell infection efficiency was evaluated. The knockdown efficiency was determined by qRT-PCR and Western blot.

Western Blotting Analysis
Proteins were extracted from RCC patient tissues or cells in ice-cold RIPA buffer containing protease inhibitors and protein concentration was determined by BCA Protein Assay Kit. Western Blot assays were performed by well-established protocols as previously described.27 Image J was used for
density analysis to quantify the intensity of the Western blotting band. The primary antibodies used in Western blotting were as follows: Gai1 (1:1000, Proteintech), β-actin (1:5000, Proteintech), Akt (1:1000, CST), p-Akt (1:1000, CST), Erk (1:1000, CST), p-Erk (1:1000, CST), S6 (1:1000, CST) p-S6 (1:1000, CST), cyclin D1 (1:2000, Proteintech), CDK4 (1:1000, Proteintech), MMP-2 (1:1000, Proteintech), MMP-9 (1:1000, Proteintech), BAX (1:4000, Proteintech), Bcl2 (1:1000, Proteintech). The secondary antibody used in Western blotting was HRP goat anti-rabbit IgG (1:5000, Proteintech) and HRP goat anti-mouse IgG (1:5000, Proteintech).

RNA Isolation and Quantitative Real-Time PCR

Total RNA was isolated from RCC patient tissues or cells lysates using Trizol kit (Qiagen, Valencia, CA, USA). cDNAs were synthesized using a Thermo-script RT kit (Life Technologies, Rockville, MD, USA). Quantitative real-time PCRs were performed in in CFX96™ Real-Time System (Bio-Rad, Hercules, CA, USA), using SYBR PCR reagent (Takara, Shiga, Japan). GADPH is standardized as a gene expression level. The sequences of primers are as follows:

- Gai1-F: GCTCAACCAAATTACATCCGCAC
- Gai1-R: ATCTCTGACCTCCACATCAAC
- CDK4-F: TGCCTGAGTCTATCACACAGA
- CDK4-R: TTGATGAGGGGAAGGAAATGC
- Cyclin D1-F: TTCGTGCCCTCAAGATGAAGG
- Cyclin D1-R: GTTCCACTTGAGCTGTTCACC
- MMP-2-F: AGACCTGGATCAGGCAAAAGAC
- MMP-2-R: TTCGTTGTACCTGTCACAGGTC
- MMP-9-F: TCGAGAATCCGAGCCGCTGT
- MMP-9-R: ATGGACCGTGCTTTCTATG
- BAX-F: CAGCTGACATGTTTCTGACGG
- BAX-R: AGCCCATGATGTGTCTGTACAG
- Bcl2-F: ATTTGTCCTCTTTGAGTTCG
- Bcl2-R: TTCCAGTACTCAGTCATCCACAG
- GAPDH-F: ACTTGGTATCGTGGAGGACTC
- GAPDH-R: GTAGAGGCAGGGATGATGTTCG

Immunohistochemistry

Renal cancer and normal tissue were used for immunohistochemistry. After baking for 60 min in a constant temperature oven at 60°C, tissue chips were dewaxed in xylene and hydrated in graded alcohols. Slides were blocked for 5 min, then primary antibody Gai1 (1:100, Proteintech), Ki67 (1:8000, Proteintech) was added for incubating at 4°C overnight. After washing, secondary antibody HRP Goat Anti-Rabbit IgG (1:200, Proteintech) was added and incubated for 2 h at room temperature. Finally, the tissue specimens were stained with diamino-benzidine and examined with microscopic. Specimens were classified into negative, positive, ++ positive, or +++ positive, based on the sum of the staining intensity (varied from weak to strong) and staining extent scores.

CCK-8 Assay

Cell proliferation was measured by CCK-8 assay (CCK-8, Dojindo, Japan). Lentivirus infected 786-O and ACHN cells in exponential growth phase were seeded onto five 96-well plates (3×10^3 cells/well) in triplicate and cultured for 1, 2, 3, and 4 days. Four hours before absorbance measuring, 10 μL of CCK-8 solution was added. The absorbance was measured at 450 nm with a microplate reader after incubated at 37°C for 2 h.

Colonial Formation Assay

Lentivirus infected cells 786-O and ACHN were seeded into 6-well plates (800 cells/well) and cultured in the RPMI-1640 or MEM medium with 20% FBS for 7 days. The colonies were fixed with 4% paraformaldehyde for 30 min. The colonies were then washed with PBS and stained with 0.1% crystal violet. Finally, The cell colonies were photographed.

Wound Healing Assay

786-O and ACHN cells (5×10^5 cells/well) were seeded on a 6-well plate. The cell layer scratches in each well were incubated with serum-free medium. Take pictures with the microscope every 6 hours, and repeat 3 times to calculate the cell migration rate of each group.

Transwell Assay

Transwell kit (8.0μm pore size polycarbonate filter) with a Matrigel overlay (BD, NJ) was used to evaluate the invasion ability of cells. 786-O cells and ACHN cells (1×10^5) with 200μL of FBS-free medium was added into the upper chamber, and 600μL of medium with 20% FBS was add into the lower chamber. After incubating for 24 hours at 37°C with 5% CO2, non-invasive cells were removed on the upper surface by a cotton swab. The invaded cells were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet and counted.
Cell Apoptosis Were Detected by Flow Cytometry

To further quantify apoptotic cells, flow cytometry (FCM) analysis was performed according to the manufacturer’s instructions. 786-O and ACHN cells were seeded in a 6-well plate and cultured for 24 hours. After centrifugation (1000×g), the cells returned to normal and were suspended in binding buffer, and stained with 5μL PE Annexin V and 5μL 7-AAD. At least 5×10³ cells/sample were collected and analyzed using an EPICS XL flow cytometer (BD LSR Fortessa, USA).

**In vivo Xenograft Experiments**

Female BALB/c nude mice aged 4–6 weeks were obtained from the Animal Research Center of

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**Figure 1** Gαi1 expression is upregulated in RCC tissues and RCC cell lines. The expression of Gαi1 mRNA (A) in RCC tumor tissues with different tumor stages and normal tissues was detected by qRT-PCR. The relative quantification was calculated by the 2^−ΔΔCt method and normalized based on GAPDH. Expression level of Gαi1 protein (B) in RCC tissues and adjacent normal tissues. Gαi1 mRNA (C) and protein expression (D) in RCC cell lines and normal epithelium cell of renal tubule HK2. IHC analysis (E) of Gαi1 in RCC and adjacent normal tissues. Results were presented as mean ± SD. *P<0.05, **P<0.01, ***P<0.001.

**Abbreviations**: N, adjacent tissues; T, RCC tissues.
Nantong University. Approximately $5.0 \times 10^6$ different 786-O cells (786-O-shNC, and 786-O-shGai1) were subcutaneously injected into the left and right sides of the nude mice armpit. The mice body weight and tumor volume were measured every other week. The tumor volume calculation formula: tumor volume (mm$^3$) = length$ \times $width$^2 \times 0.52$. All mice were euthanized at t30 days after seeding 786-O cells and tumors removed, weighed, fixed and embedded in IHC. The protocol for animal experiment was approved by the Animal Ethics Committee of Nantong University and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Statistical Analysis**

Statistical analysis between the two groups was analyzed by Student’s t-test and comparisons involving multiple groups were analyzed by two-way ANOVA using GraphPad 5.02, followed by post hoc tests. All data are expressed as the mean $\pm$ SD, and $p < 0.05$ was considered represent statistically significant. All experiments were repeated at least three times.

**Results**

**Gai1 Expression is Upregulated in RCC Tissues and RCC Cell Lines**

To investigate the role of Gai1 in RCC, the mRNA and protein expression levels were detected by qRT-PCR.
Western blot and immunohistochemistry (IHC) in a series of RCC cell lines and 43 pairs of RCC tissue samples (non-metastatic tumors and their adjacent normal tissue specimens). qRT-PCR result suggested that Gai1 mRNA expression was significantly upregulated in fresh RCC tissues (Figure 1A). Western blotting and IHC analysis showed a significant increase of Gai1 protein expression in RCC (Figure 1B and E). The expression of Gai1 in five RCC cell lines examined by qRT-PCR and Western blotting revealed that Gai1 is highly expressed in RCC cell lines (Figure 1C and D).

Gai1 Knockdown Inhibited RCC Cells Proliferation and Migration in vitro
In order to further investigate the underlying mechanism of Gai1 in promoting RCC, we knockdown Gai1 expression in

Figure 3 Gai1 knockdown inhibited RCC cells proliferation and migration in vitro. (A) The effects of Gai1 knockdown on cell proliferation of 786-O and ACHN cells were detected by CCK-8 assay. (B and E) Colony formation assay showed that RCC cell colony formation ability was significantly inhibited in Gai1 knockdown group. (C and F) Wound-healing was utilized to assess the effects of Gai1 knockdown on cell migration of RCC cells. (D and G) Transwell assays were used to detect the cell invasion in RCC cells between shNC and shGai1 group. Results were presented as mean ± SD. *P<0.05.
RCC cell line 786-O cells and ACHN cells by lentiviruses-mediated expression of control shRNA (LV-shNC) and Gai1-specific shRNA (LV-shGai1) in vitro. The mRNA and protein expression of Gai1 in 786-O and ACHN cells after virus infection was detected by Western blotting and qRT-PCR, which demonstrates efficient downregulation following infection with LV-shGai1 (Figure 2A–D).

To understand the role of Gai1 in the proliferation of RCC cells, we first performed a CCK-8 assay and a colony formation assay. CCK-8 assay demonstrated that the reduced proliferation of 786-O and ACHN cells after Gai1 knocking down when compared to control cells (Figure 3A). Also, results from the colony formation assay indicated that silencing Gai1 obviously inhibited the cell proliferation, which was demonstrated by the number of 786-O and ACHN cell colonies is significantly reduced (Figure 3B and E). Then, the cell migration of 786-O and ACHN cells was further evaluated (Figure 3C and F). The wound healing assay and transwell assay indicated that Gai1 knockdown significantly impaired the invasive and migratory capabilities of 786-O and ACHN cells (Figure 3D and G). Furthermore, significant decline of CDK4, cyclin D1, MMP-2 and MMP-9 was observed after inhibiting the function of Gai1 in 786-O and ACHN cells (Figure 4A–E).

Gai1 Knockdown Promoted Cells Apoptosis in vitro

Apoptotic dysfunction caused by dysregulation of apoptosis-related proteins plays an important role in the development of cancer.24 To determine whether Gai1 influenced RCC cell apoptosis, flow cytometry assay
was performed to detect apoptotic cells. We observed that \( G\alpha_i \) knockdown obviously promoted apoptosis of 786-O and ACHN cells, respectively (Figure 5A and C). Besides, the mRNA expression and protein expression of Bax and Bcl-2 were detected by qRT-PCR and Western blot. The result of qRT-PCR and Western blot analysis showed that the expression of Bax was up-regulated and Bcl-2 was down-regulated in the \( G\alpha_i \) knockdowning RCC cells (Figure 5B–E). These results indicated that knockdown of \( G\alpha_i \) induced apoptosis of RCC cells.

\textbf{\( G\alpha_i \) Knockdown Inhibited mTOR and Erk Signaling Pathways}

Many lines of evidence show that \( G\alpha_i \) forms a complex with RTKs (FGFR, EGFR and KGFR) and mediates the
activation of downstream Gab1-PI3K-Akt signaling pathway. To investigate the efficacy of Gai1 in the signaling pathways, we used Western blot analysis to examine the activation of these pathways. We found that knockdown of Gai1 inhibited the ratio of p-Akt/t-Akt, p-S6/t-S6 and p-Erk/t-Erk in both 786-O and ACHN cells (Figure 6A and B). These results indicate that knocking down Gai1 inhibits the Akt-mTOR and Erk-MAPK signaling pathways.

**Gai1 Knockdown Inhibited Tumor Growth in Nude Mice**

According to previous results that knockdown of Gai1 led to impaired proliferation of cancer cells, we further analyzed whether Gai1 shRNA has an effect on tumor growth in vivo. To establish mice xenograft models, 786-O cells with or without Gai1 knockdown were injected subcutaneously into nude mice respectively. Our results showed that tumor growth was significantly impaired in the group of Gai1 knockdown compared with the control group (Figure 7A and B). And knockdown of Gai1 resulted in a significant reduction in tumor weight as assessed at the end of experiment (Figure 7C and D). We found that Ki-67 staining was stronger in the control group compared to Gai1-knocking down group (Figure 7E). In summary, the above results indicated that Gai1 knockdown could inhibit RCC tumor growth in vivo.

**Discussion**

Renal cancer have no specific presenting symptoms or signs. It has been reported that approximately 30% of RCC patients have a metastatic lesion at the time of initial diagnosis.25 The clinical prognosis of patients with metastatic RCC (mRCC) is extremely poor, with a median survival of only 13 months.26 The Akt/mTOR signaling pathway plays a very important role in the occurrence and development of renal cell carcinoma; therefore, targeting mTOR and block the Akt/mTOR signaling pathway is a potential effective way to treat mRCC.27,28 mTOR inhibitors are mainly targeted at mTORC1, which suppress tumor cells growth upon treatment of a variety of cell growth factors including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF).29 The related drugs such as temsirolimus and everolimus were approved by the US FDA for the treatment of progressive metastatic RCC.30 However, most RCC patients have a shorter period of effective response to mTORC1 inhibitors, and eventually most patients acquire resistance.30,31 Therefore, there is an urgent need to find new molecular mechanisms to treat RCC and improve the prognosis of patients with metastatic RCC.

The results of this study indicate that Gai1 may be a new anti-cancer target protein to slow down the growth of RCC cells. First, Gai1 mRNA and protein are up-regulated in human RCC tissue (compared to surrounding kidney tissue) and RCC cells (compared to human normal renal tubular epithelial cells). Second, Gai1 can promote RCC cell proliferation, migration and invasion, and inhibit RCC cell apoptosis. Third, the tumor growth of Gai1 knocking down RCC cells was slower than that of control tumors. Fourth, in RCC cells, Gai1 can activate the Akt-mTOR and Erk-MAPK signaling pathways.

Gai protein is essential for EGF to activate the PI3K/Akt/mTORC1 pathway,18 while EGF and EGFR are commonly
expressed in most human cancers.\textsuperscript{32} The activation of Akt and mTOR is important for tumor proliferation, survival, and metastasis. The function of Gai1 in the progress of the cancer is crucial,\textsuperscript{33} so Gai protein is likely to become a potential new target for anti-cancer therapy. The results of immunohistochemistry in this study showed that Gai1 was highly expressed in RCC tissues, and the up-regulation of Gai1 in RCC tissues was related to tumor staging, which suggests that Gai1 plays an important role in the occurrence and development of RCC. Similar studies have shown that Gai1 is highly expressed in human glioma tissue, and the up-regulation of Gai1 in glioma tissue is related to tumor grade and Akt activation. This study showed that Gai1 can promote the proliferation, migration and invasion of 786-O and ACHN cells in vitro. The establishment of subcutaneous xenogeneic tumor formation experiments in nude mice showed that Gai1 can promote the proliferation of RCC cells.

The Akt/mTOR signaling pathway plays a vital role in the occurrence and development of renal cancer,\textsuperscript{34} and studies have shown that Gai protein is essential for EGF to activate the PI3K/Akt/mTORC1 pathway.\textsuperscript{20} Therefore, this study also explored whether Gai protein can regulate the proliferation, migration and invasion of RCC cells by activating the mTOR and Erk signaling pathway. Western

Figure 7 Gai1 knockdown inhibited tumor growth in nude mice. Subcutaneous tumor model of 786-O cells with Gai1 knockdown. (A) Gai1 knockdown led to decreased tumor volume. (B) The photos of tumors derived from shNC and shGai1 cells in nude mice. (C and D) The changes in volume weight were measured at the indicated weeks after mice were transplanted. (E) IHC analysis that Gai1 knockdown reduced Ki67 protein expression. Results were presented as mean ± SD. *P<0.05.
Blot experiments showed that knockdown of Ga\(i\) inhibited the ratio of p/t-Akt, p/t-S6 and p/t-Erk in 786-O and ACHN cells.

In conclusion, we found that Ga\(i\) is highly expressed in human RCC tissues. And our further research shows that Ga\(i\) protein can regulate the proliferation, migration and invasion of RCC cells by activating the Akt-mTOR and Erk-MAPK signaling pathways. Therefore, we conducted a preliminary discussion on the role of Ga\(i\) protein in the development of RCC, which provides a new and potential therapeutic target for the treatment of RCC.

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Disclosure
The authors declare no conflict of interests.

References
1. Chow WH, Dong LM, Devesa SS. Epidemiology and risk factors for kidney cancer. Nat Rev Urol. 2010;7(5):245–257.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69(1):7–34. doi:10.3322/caac.21551
3. Moch H, Cubilla AL, Humphrey PA, et al. The 2016 WHO classification of tumours of the urinary system and male genital organs—part a: renal, penile, and testicular tumours. Eur Urol. 2016;70(1):93–105. doi:10.1016/j.eururo.2016.02.029
4. Rhoades SK, Bilen MA. A review of papillary renal cell carcinoma and MET inhibitors. Kidney Cancer. 2019;3(3):151–161. doi:10.2333/KCA-190058
5. Tahbaz R, Schmid M, Merseburger AS. Prevention of kidney cancer incidence and recurrence: lifestyle, medication and nutrition. Curr Opin Oncol. 2018;28(1):62–79. doi:10.1097/01.cuo.2017.09.0117
6. van den Heuvel C, van Ewijk A, Zeelen C, et al. Molecular profiling of druggable targets in clear cell renal cell carcinoma through targeted RNA sequencing. Front Oncol. 2019;9:117. doi:10.3389/fonc.2019.00117
7. Qu L, Ding J, Chen C, et al. Exosome-transmitted IncARSR promotes sunitinib resistance in renal cancer by acting as a competing endogenous RNA. Cancer Cell. 2016;29(5):653–668. doi:10.1016/j.ccell.2016.03.004
8. Liu X, Yang Z, Luo X, et al. Calcium-activated nucleotide 1 silencing inhibits proliferation, migration, and invasion in human clear cell renal cell carcinoma. J Cell Physiol. 2019;234(12):22635–22647. doi:10.1002/jcp.28829
9. Pal SK, Ali SM, Yakirevich E, et al. Characterization of clinical cases of advanced papillary renal cell carcinoma via comprehensive genomic profiling. Eur Urol. 2018;73(1):71–78. doi:10.1016/j.eururo.2017.05.033
10. Atzpodien J, Buer J, Sel S, et al. [Chemoimmunotherapy in the systemic treatment of advanced renal carcinoma]. Urologe A. 1999;38(5):474–478. German. doi:10.1007/s00120050316
11. Buti S, Rovere RK, Donini M, et al. Changes in lymphocyte count induced by repeated cycles with low-dose interleukin-2 and interferon-\(\alpha\) in 146 patients with renal cell carcinoma. Tumori J. 2012;98(1):45–52. doi:10.1177/00412963129800106
12. Maroto JP, Del Muro XG, Mellado B, et al. Phase II trial of sequential subcutaneous interleukin-2 plus interferon alpha followed by sorafenib in renal cell carcinoma (RCC). Clin Transl Oncol. 2013;15(9):698–704. doi:10.1007/s12094-012-0991-z
13. Pal SK, Quinn DI. Differentiating mTOR inhibitors in renal cell carcinoma. Cancer Treat Rev. 2013;39(7):709–719. doi:10.1016/j.ctrv.2012.12.015
14. Zarrabi K, Fang C, Wu S. New treatment options for metastatic renal cell carcinoma with prior anti-angiogenesis therapy. J Hematol Oncol. 2017;10(1):38. doi:10.1186/s13045-016-0374-y
15. Downes GB, Gautam N. The G protein subunit gene family. Genomics. 1999;62(3):544–552. doi:10.1006/geno.1999.5992
16. Alessi DR, James SR, Downes CP, et al. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase B alpha. Curr Biol. 1997;7(4):261–269. doi:10.1016/S0960-9822(06)00122-9
17. Marrari Y, Crouthamel M, Annanad R, et al. Assembly and trafficking of heterotrimeric G proteins. Biochemistry. 2007;46(26):7665–7677. doi:10.1021/bi070038m
18. Holgado-Madurga M, Emler DT, Moscatello DK, et al. A Grb2-associated docking protein in EGF- and insulin-receptor signalling. Nature. 1996;379(6565):560–564. doi:10.1038/379560a0
19. Cao C, Huang X, Han Y, et al. Galphai(1) and Galphai(3) are required for epidermal growth factor-mediated activation of the Akt-mTORC1 pathway. Sci Signal. 2009;2(68):ra17. doi:10.1126/scisignal.2000118
20. Zhang YM, Zhang Z-Q, Liu -Y-Y, et al. Requirement of Galphai/3-Gab1 signaling complex for keratinocyte growth factor-induced PI3K-AKT-mTORC1 activation. J Invest Dermatol. 2015;135(1):181–191. doi:10.1016/j.jid.2014.03.026
21. Marshall J, Zhou X-Z, Chen G, et al. Antidepressive action of BDNF requires and is mimicked by Ga\(i\)/3 expression in the hippocampus. Proc Natl Acad Sci U S A. 2018;115(15):E3549–E3558. doi:10.1073/pnas.1722493115
22. Sun J, Huang W, Yang S-F, et al. Galphai1 and Galphai3 mediate VEGF-induced VEGF-R2 endocytosis, signaling and angiogenesis. Thrombosis. 2018;8(17):4695–4709. doi:10.7150/thno.26203
23. Liu -Y-Y, Chen M-B, Cheng L, et al. microRNA-200a downregulation in human glioma leads to Galphai1 over-expression, Akt activation, and cell proliferation. Oncogene. 2018;37(21):2890–2902. doi:10.1038/s41388-018-0184-5
24. Mohammad RM, Muqbil I, Lowe L, et al. Broad targeting of resistance to apoptosis in cancer. Semin Cancer Biol. 2015;35(Suppl):S78–S103.
25. Cohen HT, McGovern FJ. Renal-cell carcinoma. N Engl J Med. 2005;353(23):2477–2490. doi:10.1056/NEJMra043172
26. Lalani A-KA, McGregor BA, Albiges L, et al. Systemic treatment of metastatic clear cell renal cell carcinoma in 2018: current paradigms, use of immunotherapy, and future directions. Eur Urol. 2019;75(1):100–110. doi:10.1016/j.eururo.2018.10.010
27. Zheng B, Mao J-H, Qian L, et al. Pre-clinical evaluation of AZD-2014, a novel mTORC1/2 dual inhibitor, against renal cell carcinoma. Cancer Lett. 2015;357(2):468–475. doi:10.1016/j.canlet.2014.11.012
28. Luo J, Luo X, Liu X, et al. DUSP9 suppresses proliferation and migration of clear cell renal cell carcinoma via the mTOR pathway. Onco Targets Ther. 2020;13:1321–1330. doi:10.2147/OTT.S239407
29. Sun SY. mTOR kinase inhibitors as potential cancer therapeutic drugs. Cancer Lett. 2013;340(1):1–8. doi:10.1016/j.canlet.2013.06.017
30. Brodziak A, Sobczuk P, Bartnik E, et al. Drug resistance in papillary 
RCC: from putative mechanisms to clinical practicalities. Nat Rev 
Urol. 2019;16(1):655–673. doi:10.1038/s41585-019-0233-z
31. Husseinzadeh HD, Garcia JA. Therapeutic rationale for mTOR inhi-
bition in advanced renal cell carcinoma. Curr Clin Pharmacol. 
2011;6(3):214–221. doi:10.2174/157488410797189433
32. Sigismund S, Avanzato D, Lanzetti L. Emerging functions of the 
EGFR in cancer. Mol Oncol. 2018;12(1):3–20. doi:10.1002/1878-
0261.12155
33. Stitzlein L, Rao P, Dudley R. Emerging oral VEGF inhibitors for the 
treatment of renal cell carcinoma. Expert Opin Investig Drugs. 
2019;28(2):121–130. doi:10.1080/13543784.2019.1559296
34. Mosmann D, Park S, Hall MN. mTOR signalling and cellular 
metabolism are mutual determinants in cancer. Nat Rev Cancer. 
2018;18(12):744–757. doi:10.1038/s41568-018-0074-8