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FOXK1 promotes malignant progression of breast cancer by activating PI3K/AKT/mTOR signaling pathway

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Abstract. – OBJECTIVE: The aim of this study was to investigate the expression characteristics of forkhead box K1 (FOXK1) in breast cancer (BCa). Meanwhile, its relationship [illegible] clinicopathology and prognosis of patient[illegible] BCa was also explored.

PATIENTS AND METHODS: The expres[illegible] level of FOXK1 in 65 paired BCa tissues and [illegible]ra-cancerous tissues was dete[illegible] by qua[illegible]titative Real Time-Polymeras[illegible] Reactio[illegible] (qRT-PCR). The relationship [illegible] [illegible]XK1 expression and BCa pathol[illegible]al pa[illegible]eters as well as the prognosis o[illegible] [illegible]ts [illegible]. Meanwhile, the expressio[illegible] [illegible]a cells was detected [illegible] qRT-[illegible] Subsequently, FOXK1 knock[illegible] and over[illegible]sion models were cons[illegible] by lentivir[illegible]sfection in BCa cell [illegible]ding MCF-7 [illegible]d SKBR3). The effect [illegible] [illegible]he biological functions of BCa cells was a[illegible] using Cell Counting Kit-8 [illegible]-8), cell clo[illegible]ssay and flow cyto[illegible], respectively. Fi[illegible]y, whether the role [illegible]XK1 [illegible] achieved *via* the PI3K/AKT/mTOR [illegible]aling [illegible]hway was explored.

[illegible]S: The [illegible]T-PCR results showed that [illegible] [illegible]xpres[illegible] in BCa tissues was significa[illegible] [illegible]an that of adjacent tissues. Comp[illegible] patients with low expression of FOXK1, [illegible] pathological grading was markedly higher in those with high expression. Meanwhile, [illegible]all survival rate was remarkably lower [illegible]tients with high expression. In addition, compared with the negative control group, the proliferation ability of cells in FOXK1 knockdown group was significantly decreased, while cell apoptosis was markedly up-regulated. Besides, Western blot results revealed that silencing FOXK1 could reduce the levels of key proteins in th[illegible]K/AKT/m[illegible] signaling pathway, thereby pro[illegible] the [illegible]nant progression of BCa. Fina[illegible]-IN-1, which was the inhibitor o[illegible]AKT/mTOR signaling pathway, significantly reversed the proliferative capacity [illegible]lls in FOXK1 overexpression group, as well [illegible]ed anti-apoptotic ability.

[illegible]LUSIONS: FOXK1 expression was re[illegible]markably increased both in BCa tissues and cells. Meanwhile, it was markedly associated with pathological stage and poor prognosis of patients. Besides, FOXK1 might promote the malignant progression of BCa by inhibiting the PI3K/AKT/mTOR signaling pathway.

Key Words:
FOXK1, PI3K/AKT/mTOR, Breast cancer (BCa), Malignant progression.

Introduction

Breast cancer (BCa), as a common tumor threatening human health, has an increasing trend of morbidity and mortality year by year[1,2]. The latest statistical research led by the United States has shown that BCa ranks first among women in the United States and most parts of the world. Meanwhile, the mortality of BCa ranks second among all female cancer patients[3,4]. According to the statistics of the annual tumor registration report released by China Cancer Registry, the incidence of BCa ranks first among all female malignant tumors in China[4-6]. The main cause of death in BCa patients is the rapid

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growth of BCa cells and other organs, as well as even systemic metastasis. This results from the proliferation, migration and invasiveness of BCa cells[7]. Therefore, elucidating the molecular mechanism of BCa helps to demonstrate the mechanism of cell proliferation, migration and invasion. It is of great significance for clinical evaluation of malignancy and prognosis, which is also important for gene-targeted therapy of BCa[7]. Currently, significant breakthroughs have been made in drug susceptibility and surgical techniques. However, the recurrence and metastasis of BCa still occur frequently, which is the leading cause of death in patients with advanced BCa[8,9]. Furthermore, early diagnosis and treatment can greatly reduce the further occurrence of tumor deterioration. Therefore, scientists have recently paid more attention to exploring potential molecular markers for recurrence and metastasis, which may provide new directions for BCa treatment[9].

The forkhead box (FOX) protein family is a kind of transcription factors whose DNA binding region has a winged helix structure. There are about 19 subfamilies of the FOX protein family. Recent studies have shown that the FOX protein family regulates the transcriptional activity of target genes by binding DNA. Meanwhile, it also participates in the regulation of cell signal transduction, cell cycle regulation and metabolism. Kwak et al[10] have demonstrated that it plays a role in various biological processes, such as embryonic development [illegible] immune regulation. Forkhead box K1 (FOXK1) is a member of the FOX family of transcription factors. It is a transcriptional repressor of downstream [illegible], such as FOX04, Mef2, P21 and [illegible]B[11,12]. [illegible] et al[10] have demonstrated that the FOX transcription factor family is closely correlated with malignancy and embryogenesis. FOXK1 is overexpressed in various tumors, including esophageal cancer, pancreatic [illegible]ancer [illegible]nwhile, the expression level of FOXK1 is [illegible]nt in gastrointestinal tumors. The[illegible]se FOX proteins exert a biphasic regulat[illegible] influence on cell growth, proliferation and differentiation[13-16]. In colorectal cancer, lym[illegible]nd glioblastoma cell lines, protein kinase B (AKT) acts as a downstream regulator of phosphatidil inositol 3-kinase (P13K). AKT activates cell cycle by phosphorylating FOXO1, FOX03 and FOX04, thereby facilitating the process of tumor cell transformation. Dephosphorylated (activated) FOXOI, FOX03 and FOX04 can be transferred into the nucleus to induce cell dormancy or apoptosis[12,17]. The PI3K-AKT-mTOR signal transduction pathway controls a large number of tumor markers. All of them are involved in important cellular functions, including cell cycle, cell survival, metabolism, cell movement and genomic instability[18-20]. Thus, scholars[21,22] have proved that the phosphorylation of the PI3K-AKT-mTOR signal transduction pathway is closely associated with the occurrence and development of various human tumors.

AKT and mTOR are included in intracellular signaling pathways activated by [illegible]. However, whether FOXK1 regulates the PI3K-AKT-mTOR signal transduction pathway in BCa cells has not been fully elucidated in domestic and foreign studies[21,22]. In this work, we investigated whether FOXK1 was a serological marker for early diagnosis of BCa, and whether it could provide a possible option for target gene therapy. Therefore, we proposed that FOXK1 might participate in the process of BCa cell proliferation and apoptosis by targeting the PI3K/AKT/mTOR signaling pathway.

Patients and Methods

Patients and BCa Samples

A total of 65 patients who received treatment in the general surgery and oncology departments in our hospital were enrolled in this study. Paired BCa tissues and para-cancerous were obtained from fresh specimens of biopsy or surgical resection. All collected tissue samples were stored in a refrigerator at -80°C for subsequent use. 65 patients with BCa aged from 30 to 78 years, with an average of 64.9 (56.9-79.8) years, were diagnosed by two senior directors of pathologists for confirmation. This study was approved by the Ethics Committee of Sanya People's Hospital. Signed informed consents were obtained from all participants before the study.

Cell Lines and Reagents

Three human BCa cell lines (MCF-7, MDA-MB-231 and SKBR3) and one normal mammary epithelial cell line (MCF-10A) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Both Dulbecco's Modified Eagle's Medium (DMEM) medium and fetal bovine serum (FBS) were purchased from Life Technologies (Gaithersburg, MD, USA). All cells were cultured in high glucose DMEM medi-

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um containing 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL), and maintained in an incubator at 37°C with 5% CO_2. When grown to 80% - 90% of confluence, the cells were digested with 1 × trypsin + ethylenediamine tetraacetic acid (EDTA).

Cell Transfection

Negative control (NC) and FOXK1 (FOXK1 or FOXK1-S) containing FOXK1 lentiviral sequence were purchased from Shanghai Jima Company (Shanghai, China). Cells were first seeded into 6-well plates and cultured to a cell density of 70%. Subsequently, lentiviral transfection was performed according to the manufacturer's instruction. 48 hours after transfection, the cells were selected for quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) analysis, Western blot analysis and cell function experiments.

Cell Proliferation Assay

The proliferation of cells was examined using Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan). Briefly, 10[illegible] of cell suspension (including 2000 cells[illegible] added in each well, followed by the add[illegible] of 10 μL of CCK-8 solution. Afterward, [illegible] cells were incubated for 1 h in [illegible]cubator [illegible] 37°C. Finally, optical density [illegible]orbanc[illegible] of each well at 450 nm w[illegible]tect[illegible]y a microplate reader.

Colony Formatio[illegible]ssay

48 h after tra[illegible]n, the ce[illegible] first collected. 200 ce[illegible] seeded into [illegible]l plates, followed by [illegible]omplete medium for two weeks. The [illegible]ture[illegible]m was changed after the fi[illegible]eek, and th[illegible]ged twice a week. Aft[illegible]o weeks, the c[illegible]were washed twice [illegible]Phos[illegible]te-Buffered Saline (PBS; Gibco, [illegible]d I[illegible], NY, USA). Subsequently, the cells [illegible] with [illegible] of methanol for 20 minutes a[illegible]ed [illegible]0.1% crystal violet staining solu[illegible]inutes. Finally, cells were photographe[illegible]r a light-selective environment, and the numbe[illegible] of formed colonies was counted.

[illegible] Cytometry Analysis of Cell Apoptosis

Cell apoptosis was detected by flow cytometry, combining with Annexin V-FITC (fluorescein isothiocyanate; Millipore, Billerica, MA, USA) and Propidium Iodide (PI). A specific procedure was as follows. First, the density of cells was adjusted to about 1×10^6 cells/ml. The medium was removed, and cells were washed twice with PBS. Secondly, the cells were re-suspend[illegible] gently with 0.5 mL of pre-cold 1× binding [illegible]fer. 1.25 uL of Annexin V-FITC was [illegible] followed by incubation at room temperatu[illegible] light-proof reaction for 15 min. Thi[illegible] the c[illegible] were centrifuged at 1000 × g fo[illegible]in at room temperature, and the superna[illegible]was [illegible]ved. Lastly, the cells were re-su[illegible]d [illegible]y with 0.5 mL of pre-cooled 1 × b[illegible]ffer, [illegible] 10 uL PI was added. [illegible]amp[illegible]e [illegible] on ice and stored i[illegible]dark, fol[illegible]ow cytometry (BD B[illegible]es, Frankli[illegible]s, NJ, USA) analysis[illegible]

Quantitative Real Ti[illegible]merase Chai[illegible]ion

T[illegible]RNA in BCa cell lines and tissues was ex[illegible]ed by TR[illegible] reagent (Invitrogen, Carlsba[illegible], USA). T[illegible] extracted RNA was reverse tra[illegible]d into [illegible]first strand of cDNA using Prim[illegible]agent (TaKaRa, Otsu, Shiga, Japan). Q[illegible]ative Real Time-Polymerase Chain [illegible]tion (qRT-PCR) reaction was performed us[illegible]® Premix Ex TaqTM (TaKaRa, Otsu, [illegible]nd StepOne Plus Real Time-PCR System (Applied Biosystems, Foster City, CA, USA). The primers used were as follows: FOXK1: forward: 5'-TGAGCCCCAGAACTGAGAAG-3', reverse: 5'-GCGCTACACTGACATTGGAG-3'; β-actin: forward: 5'-CCTGGCACCCAGCACAAT-3', reverse: 5'-TGCCGTAGGTGTCCCTTTG-3'. Data analysis was performed using ABI Step One software (Applied Biosystems, Foster City, CA, USA). Finally, the relative expression levels of mRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method.

Western Blot Assay

Transfected cells were first lysed with cell lysis buffer, shaken on ice for 30 minutes and centrifuged at 14,000 × g for 15 minutes at 4°C. Total protein concentration was calculated by the bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Waltham, MA, USA). Extracted proteins were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Western blot analysis was performed according to standard procedures. Primary antibodies included FOXK1, PI3K, AKT, mTOR, GAPDH and secondary antibodies were anti-mouse and

anti-rabbit. All of the antibodies used were purchased from Cell Signaling Technology (Danvers, MA, USA).

Statistical Analysis

GraphPad Prism 5 V5.01 software (La Jolla, CA, USA) was used for all statistical analysis. Student's *t*-test was used to compare the difference between the two groups. One-way ANOVA was performed to compare the difference among different groups, followed by Post-Hoc Test (Least Significant Difference). Independent experiments were repeated at least three times. Experimental data were represented as averaged ± standard deviation. There were three levels of $p<0.05$, $p<0.01$ and $p<0.001$ as the significance level, and $p<0.05$ was considered statistically significant.

Results

FOXK1 Was Highly Expressed in BCa Tissues and Cell Lines

To determine the role of FOXK1 in BC[illegible] first collected 65 pairs of BCa tissues [illegible] ra-cancerous tissues. The differences of F[illegible] expression in BCa tissues and pa[illegible]ancerous tissues were detected by qRT-P[illegible]he results showed that FOXK1 level w[illegible]gnific[illegible] elevated in BCa tissues whe[illegible]ar[illegible]th para-cancerous tissues (Figure [illegible]sugges[illegible] that FOXK1 might act [illegible]mo[illegible]tin[illegible] in BCa. In addition [illegible]ound that [illegible]vel in patients with T[illegible]ge was mark[illegible]higher than in those [illegible]e (Figure 1B). At the same time, the [illegible]pressi[illegible] of FOXK1 in BCa cell lines was detected. T[illegible]ts demonstrated that F[illegible] expression in [illegible]a cells was con-

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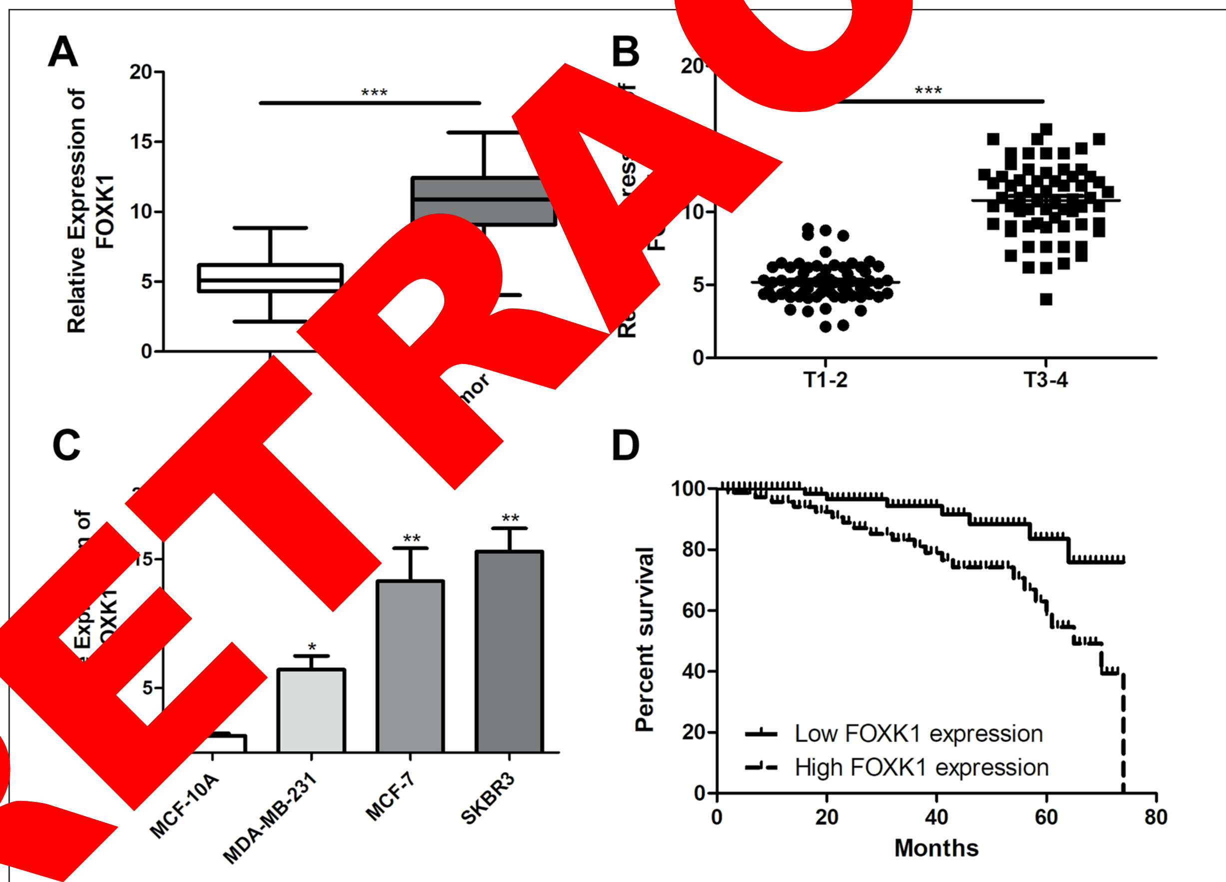

Figure 1. FOXK1 was highly expressed in BCa tissues and cell lines. **A,** QRT-PCR detection of FOXK1 level in BCa tissues and adjacent tissues. **B,** QRT-PCR detection of BCa tumor histo-pathological grades between T1-2 and T3-4 level of FOXK1. **C,** QRT-PCR was used to detect the level of FOXK1 in BCa cell lines. **D,** Kaplan-Meier survival curve of BCa patients based on FOXK1 level. The prognosis of patients with higher level of FOXK1 was significantly worse than that of patients with lower level. Data were represented as mean ± SD. $*p<0.05$, $**p<0.01$, $***p<0.001$.

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spicuously higher than that of normal breast cell line MCF-10A. Among them, MCF-7 and SKBR3 cell lines expressed the highest level of FOXK1 (Figure 1C).

FOXK1 Level Was Correlated With Clinical Stage and Overall Survival of BCa Patients

According to qRT-PCR results in 65 pairs of BCa tissues and para-cancerous tissues, all patients were divided into high FOXK1 expression group and low FOXK1 expression group, respectively. The number of patients in each group was counted. Subsequently, the relationship between FOXK1 expression and age, gender, pathological stage, lymph node metastasis and distant metastasis of BCa patients was analyzed by Chi-square test. As shown in Table I, a high level of FOXK1 was positively correlated with pathological stage of BCa. In addition, to explore the relationship between the expression level of FOXK1 and the prognosis of BCa patients, we collected relevant follow-up data. The Kaplan-Meier survival curves showed that high expression of FOXK1 was significantly associated with poor prog[illegible] of BCa patients. Higher FOXK1 level ind[illegible] worse prognosis of patients (p<0.05; Figure [illegible]

Knockdown of FOXK1 Inh[illegible]e Proliferation of BCa Ce[illegible]

To explore the effects o[illegible]K1 o[illegible]ifer-ation of BCa cells, the FO[illegible]

was first successfully constructed. Transfection efficiency was verified by qRT-PCR and Western blot assay (Figure 2A, 2B). Subsequently, the proliferation of cells in the NC group and FOXK1 i[illegible]terference group FOXK1-S was detected by C[illegible] 8 and colony formation assay. The results[illegible] that the proliferation rate of cells in FO[illegible] group was significantly decreased w[illegible]ompa[illegible] with the NC group (Figure 2C, 2D[illegible]).

Knockdown of FOXK1 In[illegible] poptosis of BCa Ce[illegible]

To explore the ef[illegible]f FOXK[illegible]op-tosis of BCa cel[illegible]rst transfect[illegible]C and FOXK1-S (F[illegible]rence) sequence into BCa cells. Ce[illegible]popto[illegible] measured by flow cytometry. Annexin V-F[illegible] double staining result[illegible] that the apo[illegible]s rate of cells in the [illegible]K1-S group was remarkably higher than th[illegible] the NC gr[illegible] (Figure 2F).

Kno[illegible]OXK1 Decreased the Lev[illegible]I3K/AKT/mTOR [illegible]aling Pathway

[illegible] blot assay was used to further ex-[illegible] mechanism in which FOXK1 promot-[illegible]d the malignant progression of BCa. The results showed that the protein levels of p-PI3K, p-AKT and p-mTOR in the PI3K/AKT/mTOR pathway were markedly increased after silencing of FOXK1 (Figure 3).

Table I. Asso[illegible]K1 expression [illegible]n clinicopathologic characteristics of breast cancer.

[illegible]ameters	[illegible]mber of cases	FOXK1 expression		p-value
		Low (%)	High (%)	
[illegible]e (yea[illegible]				0.673
[illegible]0	30	17	13	
[illegible]	35	18	17	
[illegible]				0.783
M[illegible]	25	14	11	
Fema[illegible]	40	21	19	
T stage				0.018
[illegible]T2	32	22	10	
[illegible]	33	13	20	
Ly[illegible]ph node metastasis				0.297
No	28	13	15	
Yes	37	22	15	
Distance metastasis				0.128
No	39	24	15	
Yes	26	11	15	

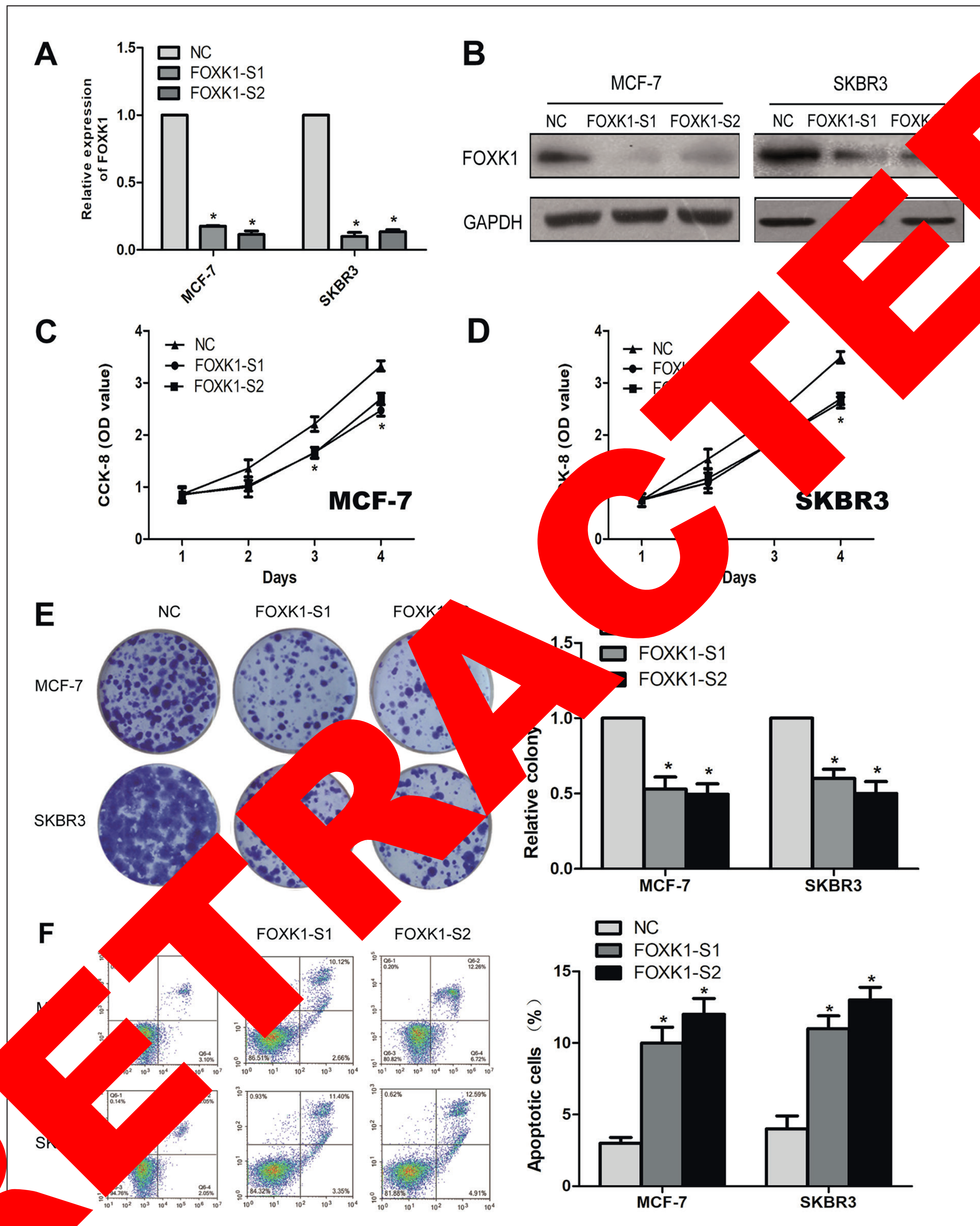

Figure 2. Knockdown of FOXK1 inhibited the proliferation and apoptosis of BCa cells. **A,** QRT-PCR verified the interference efficiency of small interference transfection of FOXK1 in MCF-7 and SKBR3 cell lines. **B,** Western blot verified the interference efficiency after small interference transfection of FOXK1 in MCF-7 and SKBR3 cell lines. **C, D,** CCK-8 assay detected the effect of FOXK1 on the proliferation of BCa cells (MCF-7 and SKBR3). **E,** Colony formation assay detected the proliferation of MCF-7 and SKBR3 cell lines after interference with FOXK1 (Magnification × 40). **F,** Flow cytometry assay detected the effect of interference with FOXK1 on the apoptosis of BCa (MCF-7 and SKBR3). Data were represented as mean ± SD. *p<0.05.

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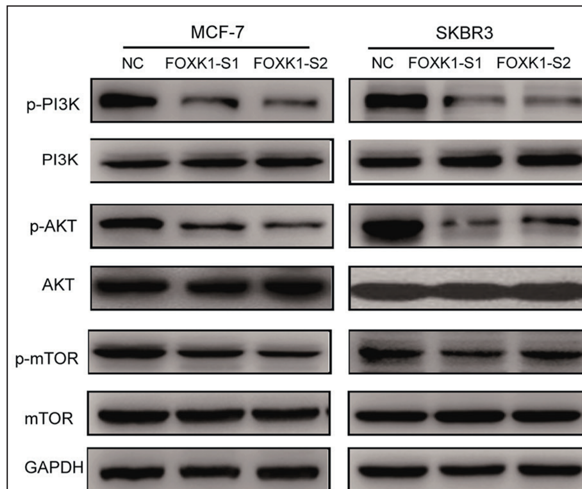

Figure 3. The mechanism of FOXK1 in the regulation of the PI3K/AKT/mTOR signaling pathway in BCa cells. Western blotting verified the protein expression levels of PI3K, AKT and mTOR after interference with FOXK1 in MCF-7 and SKBR3 cell lines.

PI3K/AKT/mTOR Signaling Pathway Inhibitor PI3KĐ/mTOR-IN-1 Reversed [illegible] FOXK1 Induced Carcinogenesis

To further explore the mechanism of FOX[illegible] BCa, we successfully constructed FOXK1 o[illegible] expression model. Then, the PI[illegible]KT/mTO[illegible] pathway inhibitor PI3Kα/mTO[illegible]s added and FOXK1 level was dete[illegible]y q[illegible]CR and Western blot assay (Figu[illegible]4B)[illegible]nt-ly, CCK-8 and colony for[illegible] formed to detect th[illegible]lifer[illegible]f cells in the FOXK1 group a[illegible]XK1+PI[illegible]TOR-IN-1 group. Meanw[illegible]w cytometry[illegible]pplied to detect cell a[illegible]a result, the proliferation rate of cells[illegible]FOX[illegible]Kα/mTOR-IN-1 group was si[illegible]ficantly decr[illegible]hen compared with the [illegible]K1 group (Figu[illegible], 4D, 4E). Annexin V[illegible]C/PI[illegible]uble staining results showed that [illegible]po[illegible] rate of cells in the FOXK1+PI3Kα/[illegible]1 gro[illegible]s markedly higher than that o[illegible]XK[illegible]p (Figure 4F).

Discussion

[illegible] recent years, due to changes in dietary structure and lifestyle, as well as the combination of environmental and biological factors, the incidence of BCa has shown an upward trend[1-3]. Currently, BCa is one of the most common malignant tumors among women, which is also a major risk for cancer-related deaths[2-4]. Rapid progression is the leading cause of death of BCa patients, accounting for more than 90% of deaths[5,6]. Researchers[6-8] have found that malignant gr[illegible] and active cells can enter the body's circul[illegible] system before the diagnosis of tumors, [illegible]ing the importance of early diagnosis of [illegible] metastasis. Therefore, searching f[illegible]olecu[illegible] markers related to proliferation [illegible]greatly improve the diagnosis rate of B[illegible] patie[illegible] This can also provide effective a[illegible]ibl[illegible]atment strategies[6-9].

The forkhead (FOX[illegible]e fa[illegible] c[illegible] transcription factor[illegible]encode a[illegible]proteins. These prot[illegible]rt regulatory[illegible]ctions, which are inv[illegible]aintenance of embryonic developm[illegible] and [illegible]ic balance[10-12]. The structural characteristic o[illegible]ranscription factors i[illegible]s a highly co[illegible]ed DNA binding do[illegible]n evolution, namely winged domain or fo[illegible]d domain[10]. [illegible]XK1 is a sequence-specific D[illegible]nding pr[illegible] binding to the myoglobin CC[illegible] seq[illegible]. Studies[12-15] have indicated that [illegible] known as myocyte nuclear factor o[illegible]) is located on chromosome 5 in [illegible]e, consisting of 9 sub-component. In the [illegible]OXK1 at the amino acid level, human [illegible] gene (consisting of 733 amino acids) and mouse FOXK1 gene (consisting of 791 amino acids) have 88.7% homoousia[16]. At present, people have gained some understanding of the promotional effects of FOXK1 on the development and progression of tumors. There are preliminary reports on FOXK1 in colorectal cancer, intestinal cancer and osteosarcoma. However, the exact role of FOXK1 in BCa remains unclear[13-16]. In BCa cells, transcription factors of FOXK1 and FOXK2 are DVL (Dishevelled)-related proteins. These two transcription factors can promote DVL nuclear translocation and activate the Wnt/b-catenin signaling pathway. Meanwhile, FOXKI and FOXK2 are highly expressed in human BCa tissues[15,16]. Furthermore, it has been found[16] that the deletion of FOXK1 and FOXK2 can simultaneously inhibit the Wnt pathway, cell proliferation and *in situ* implanted tumors.

Nowadays, abnormal expression of FOXK1 has been found in a variety of tumors of different organs of the body. This suggests[12-14] that it may be associated with malignant progression of tumors. In our work, we collected a large number of clinical BCa samples for the first time to explore the role of FOXK1 in BCa development. The expressions of FOXK1 in fresh BCa surgical

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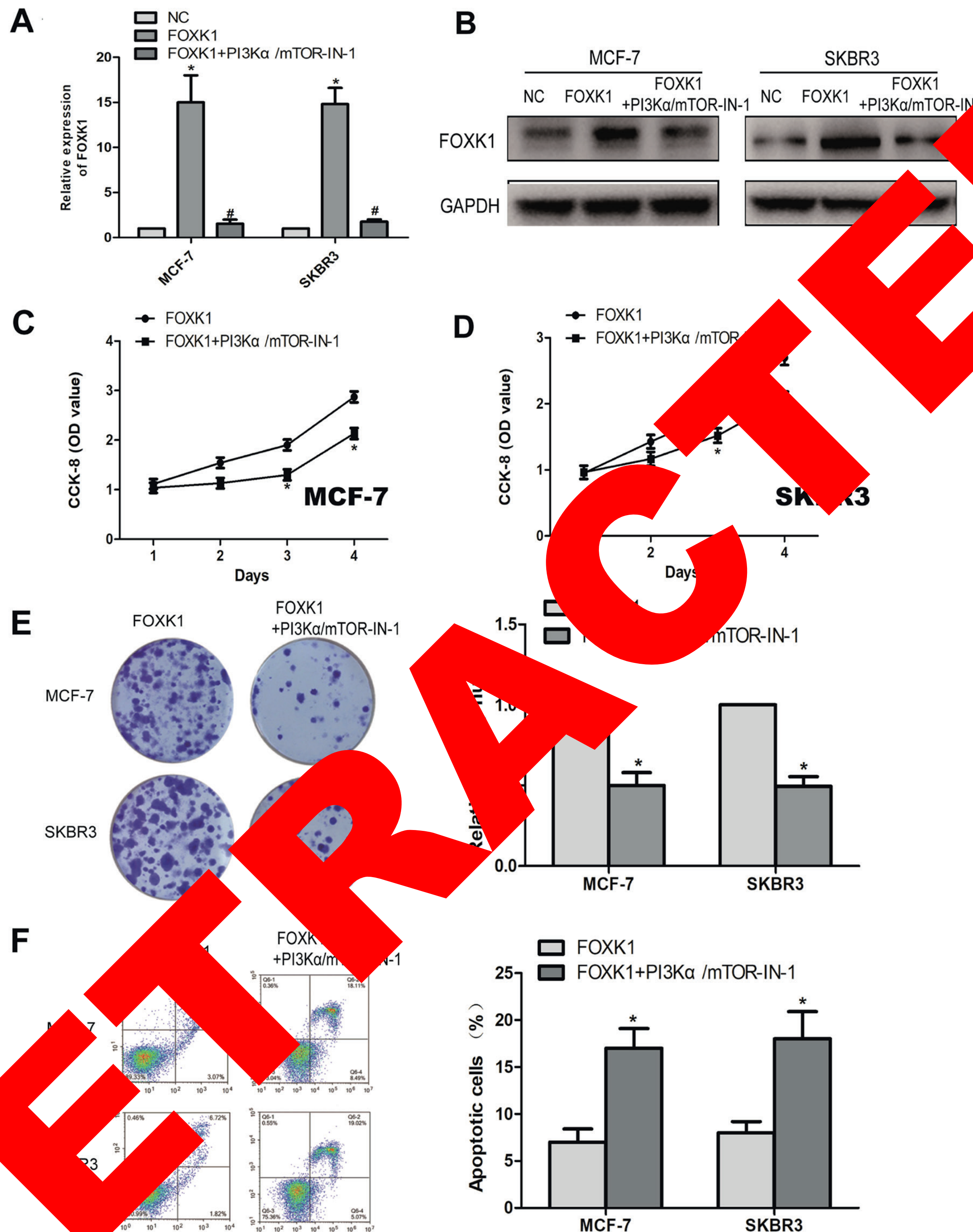

[illegible] 4. PI3K/AKT/mTOR pathway inhibitor PI3Kα/mTOR-IN-1 reversed the malignant progression of BCa cells induced by [illegible]XK1. **A,** QRT-PCR verified the expressions of FOXK1 in MCF-7 and SKBR3 cell lines of FOXK1 overexpressing and FOXK1+PI3Kα/mTOR-IN-1 groups. **B,** Western blot revealed the protein expression of FOXK1 in MCF-7 and SKBR3 cell lines of FOXK1 overexpressing and FOXK1+PI3Kα/mTOR-IN-1 groups. **C, D,** CCK-8 assay demonstrated that PI3Kα/mTOR-IN-1 reversed the effect of FOXK1 on the proliferation of BCa cells. **E,** Colony formation assay revealed the effect of PI3Kα/mTOR-IN-1 on the proliferation of MCF-7 and SKBR3 cell lines (Magnification × 40). **F,** Flow cytometry was performed to detect the effect of FOXK1+PI3Kα/mTOR-IN-1 on the apoptosis of MCF-7 and SKBR3 cell lines. Data were represented as mean ± SD. $^*p<0.05$.

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specimens and BCa cell lines were detected at both transcriptional and protein levels. The results showed that the expressions of FOXK1 were significantly increased in most BCa tissues and BCa cell lines when compared with para-cancerous tissues and normal BCa cell line MCF-10A, respectively. The above experimental results indicated that a high level of FOXK1 played an extremely important role in the development and progression of BCa. To further understand the effects of FOXK1 on the biological behaviors of BCa cell lines, CCK-8 cell proliferation assay, colony formation assay and flow cytometry were performed in BCa cells overexpressing and down-expressing FOXK1. We found that FOXK1 markedly promoted the proliferation of BCa cells and inhibited cell apoptosis, thereby playing an important role in BCa. However, the specific molecular mechanism remained unclear.

As a signal transduction pathway, the PI3K/AKT/mTOR signaling pathway is widely involved in cell growth, proliferation and differentiation regulation. PI3K plays an important role in the development, progression, treatment and outcome of malignant tumors[17]. The PI3K s[illegible] transduction pathway has also been recogni[illegible] an important pathway for BCa research for r[illegible] years[23]. Besides, AKT, protein kinase B (PKB[illegible] a Ser/Thr protein kinase that is c[illegible] related [illegible] the development of malignant [illegible] is also [illegible] downstream effector of PI3[illegible] AK[illegible] considered as a key link in the P[illegible] KT/[illegible] al transduction pathway, whi[illegible] phorylation. Activa[illegible] AKT[illegible] tes a series of cellular physi[illegible] l proces[illegible] egulating substrate prot[illegible] ltiple malig[illegible] ells have been confi[illegible] vate AKT abnormally[24]. Therefore, [illegible] expr[illegible] of the occurrence and regula[illegible] mechanism[illegible] 3K/AKT/mTOR is ess[illegible] to search for n[illegible] argets for the treat[illegible] of BC[illegible] specially tumor cell metastasis. In [illegible] curr[illegible] ork, to understand whether FOXK1 [illegible] the de[illegible] ment of BCa by regulating th[illegible] AK[illegible] OR signaling pathway, Western [illegible] formed to detect the expression levels o[illegible] proteins in the PI3K/AKT/mTOR signaling pathway after knockdown of FOXK1. [illegible] lts showed that the expression levels of k[illegible] proteins, including PI3K, AKT and mTOR, were remarkably decreased after knock-down of FOXK1. This indicated that FOXK1 might play an important role in promoting the proliferation and inhibiting the apoptosis of BCa. Subsequently, we used the pathway inhibitor PI3Kα/mTOR-IN-1 to further verify whether FOXK1 promoted the malignant progression of BCa through this signaling pathway. It was found that PI3Kα/mTOR-IN-1 could successfully reverse the pr[illegible] moting effect of BCa caused by FOXK1. Wi[illegible] progress of research, further understandi[illegible] roles of specific genes in the biological fu[illegible] and the development of tumors will [illegible] ore c[illegible] ducive to the diagnosis, treatmen[illegible] prognosis of tumors.

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FOXK1 expre[illegible] s significant[illegible] eased in BCa tissue[illegible] s. Meanwhile, its expression was a[illegible] ciate[illegible] athological stages and poor prognosis of B[illegible] ddition, FOXK1 might [illegible] e the prolifera[illegible] and inhibit cell apo[illegible] s of BCa by regulating the PI3K/AKT/m[illegible] signaling [illegible] way.

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The Authors [illegible] re that they have no conflict of interests.

References

1) Yeo SK, Guan JL. Breast cancer: multiple subtypes within a tumor? Trends Cancer 2017; 3: 753-760.
2) D'Angelo A, Rinaldi P, Belli P, D'Amico R, Carlino G, Grippo C, Giuliani M, Or-landi A, Infante A, Manfredi R. Usefulness of automated breast volume scanner (ABVS) for monitoring tumor response to neoadjuvant treatment in breast cancer patients: preliminary results. Eur Rev Med Pharmacol Sci 2019; 23: 225-231.
3) DeSantis CE, Fedewa SA, Goding Sauer A, Kramer JL, Smith RA, Jemal A. Breast cancer statistics, 2015: convergence of incidence rates between black and white women. CA Cancer J Clin 2016; 66: 31-42.
4) Stamatovic L, Susnjar S, Gavrilovic D, Minic I, Ursulovic T, Dzodic R. The influ-ence of breast cancer subtypes on the response to anthracycline neoadjuvant chemo-therapy in locally advanced breast cancer patients. J BUON 2018; 23: 1273-1280.
5) Zhao J, Jiang GQ. MiR-4282 inhibits proliferation, invasion and metastasis of hu-man breast cancer by targeting Myc. Eur Rev Med Pharmacol Sci 2018; 22: 8763-8771.
6) Hong W, Dong E. The past, present and future of breast cancer research in China. Cancer Lett 2014; 351: 1-5.
7) Nagini S. Breast cancer: current molecular therapeutic targets and new players. Anti-cancer Agents Med Chem 2017; 17: 152-163.

8) Cimino-Mathews A, Foote JB, Emens LA. Immune targeting in breast cancer. On-cology (Williston Park) 2015; 29: 375-385.
9) de la Mare JA, Contu L, Hunter MC, Moyo B, Sterrenberg JN, Dhanani KC, Mutsvunguma LZ, Edkins AL. Breast cancer: current developments in molecular approaches to diagnosis and treatment. Recent Pat Anticancer Drug Discov 2014; 9: 153-175.
10) Kwak HJ, Ryu KB, Medina Jimenez BI, Park SC, Cho SJ. Temporal and spatial expression of the Fox gene family in the Leech Helobdella austinensis. J Exp Zool B Mol Dev Evol 2018; 330: 341-350.
11) Zhang H, Wu X, Xiao Y, Wu L, Peng Y, Tang W, Liu G, Sun Y, Wang J, Zhu H, Liu M, Zhang W, Dai W, Jiang P, Li A, Li G, Xiang L, Liu S, Wang J. Coexpres-sion of FOXK1 and vimentin promotes EMT, migration, and invasion in gastric cancer cells. J Mol Med (Berl) 2018; 97: 163-176.
12) Cui H, Gao Q, Zhang L, Han F, Wang L. Knockdown of FOXK1 suppresses liver cancer cell viability by inhibiting glycolysis. Life Sci 2018; 213: 66-73.
13) Ji ZG, Jiang HT, Zhang PS. FOXK1 promotes cell growth through activating wnt/beta-catenin pathway and emerges as a novel target of miR-137 in glioma. Am J Transl Res 2018; 10: 1784-1792.
14) Huang X, Xiang L, Li Y, Zhao Y, Zhu H, Xiao [illegible] M, Wu X, Wang Z, Jiang P, Qing H, Zhang Q[illegible] Zhang W, Li A, Chen Y, Liu S, Wang J. Snail/F[illegible] Cyr61 signaling axis regulates the epithelial-[illegible]enchymal transition and metastasis in colore[illegible] cancer. Cell Physiol Biochem 20[illegible]: 590-60[illegible]
15) Ma X, Yang X, Bao W, Li S, L[illegible] Zhao [illegible] Wang J, Zhao C. Circular [illegible]circ[illegible]B2 facilitates lung cancer cell [illegible]ration[illegible]nvasion via miR-1275/FOXK1 a[illegible] Commun 2018; 49[illegible] 100[illegible]
16) Xu H, Huang S, [illegible] Zhang [illegible]g X. FOXK1 promotes gli[illegible]ma pro-life[illegible]d metastasis throu[illegible]tion of Snail tr[illegible]ption. Exp Ther Me[illegible]08-3116.
17) Zhang P, Tang WM, Zhang H, Li YQ, Peng Y, Wang J, Liu GN, Huang XT, Zhao JJ, Li G, Li AM, Bai Y, Chen Y, Ren YX, Li GX, Wang YD, Liu SD, Wang JD. MiR-646 inhibited cell proliferation and EMT-induce[illegible] metastasis by targeting FOXK1 in gastric can[illegible] Br J Cancer 2017; 117: 525-534.
18) Asati V, Mahapatra DK, Bharti SK. PI3K/A[illegible] and Ras/Raf/MEK/ERK signaling pathways[illegible] itors as anticancer agents: structu[illegible] and p[illegible] macological perspectives. Eur J [illegible]hem 201[illegible] 109: 314-341.
19) Guerrero-Zotano A, Mayer [illegible]rteag[illegible] PI3K/AKT/mTOR: role in breas[illegible]gressio[illegible] drug resistance, and t[illegible]atme[illegible]r Meta[illegible]sis Rev 2016; 35: 5[illegible].
20) Duan S, Huang W[illegible]X, Liu X, Che[illegible], Hu Y, Song W, Z[illegible]PDH2 promo[illegible]olorectal cancer [illegible]rough activation of the PI3K/AKT[illegible]R a[illegible]/AKT/FOXO1 signaling pathways. J Exp [illegible]cer Res 2018; 37: 304[illegible]
21) [illegible]ichetti F, Raimondi [illegible], Niger M, Prinzi N, [illegible]chio M, Tambo[illegible]ni E, Perrone F, Pruneri G, Di Bar[illegible]omeo M, [illegible]d F, Pusceddu S. Targeting the [illegible]K/AKT/mT[illegible]athway in biliary tract cancers: [illegible]w of cu[illegible] evidences and future perspec[illegible]at Rev 2019; 72: 45-55.
22) Gas[illegible] Besharat ZM, Farooqi AA, Khalid S, Taghavi K, Besharat RA, Sabato C, Papadia A, Panici [illegible] Mueller MD, Ferretti E. MiRNAs and their in[illegible]y with PI3K/AKT/mTOR pathway in ovari[illegible]cancer cells: a potential role in platinum resistance. J Cancer Res Clin Oncol 2018; 144: 2313-2318.
23) Sanchez-Alegria K, Flores-Leon M, Avila-Munoz E, Rodriguez-Corona N, Arias C. PI3K signaling in neurons: a central node for the control of multiple functions. Int J Mol Sci 2018; 19: 3725.
24) Shao S, Nie M, Chen C, Chen X, Zhang M, Yuan G, Yu X, Yang Y. Protective action of liraglutide in beta cells under lipotoxic stress via PI3K/Akt/FoxO1 pathway. J Cell Biochem 2014; 115: 1166-1175.

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