Am J Transl Res 2021;13(5):4152-4166
www.ajtr.org /ISSN:1943-8141/AJTR0124741

Original Article

KIF22 promotes progress of esophageal squamous cell carcinoma cells and is negatively regulated by miR-122

Jian Wang[2*], Peng-Yi Yu[3*], Jing-Ping Yu[1], Ju-Dong Luo[1], Zhi-Qiang Sun[1], Fei Sun[1], Ze Kong[1], Jian-Lin Wang[1]

[1]Department of Radiotherapy, The Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University, Changzhou 213003, Jiangsu Province, China; [2]Department of Radiotherapy, Jiangyin People's Hospital, Jiangyin 214400, Jiangsu Province, China; [3]Department of Cardiothoracic Surgery, The Third Affiliated Hospital of Soochow University, Changzhou 213003, Jiangsu Province, China. [*]Equal contribution.

Received October 22, 2020; Accepted March 14, 2021; Epub May 15, 2021; Published May 30, 2021

Abstract: Esophageal squamous cell carcinoma (ESCC) increases at fast rate of all cancer types in China, which urges the investigations of its potential mechanism. In this research, a highly expressed kinesin superfamily protein 22 (KIF22) was founded both in ESCC tissues and cancer cell lines. The following experiments pointed out that down-regulation of KIF22 remarkably restrained the malignant progression of ESCC cells. Besides, KIF22 knockdown promoted ESCC cells apoptosis and arrested cells in G0/G1 phase, while KIF22 also regulated the expression of cell cycle- and EMT-related proteins. Previous research revealed that the aberrant expressions of microRNAs (miRNAs) are related to tumors development. Based on the predict result, KIF22 was considered as the target of miR-122, which was demonstrated by luciferase reporter assay. miR-122 inhibitor could significantly reverse the function of KIF22 knockdown, including cell proliferation, migration and invasion. Furthermore, down-expressed miR-122 altered the function of KIF22 knockdown on cell cycle- and EMT-related proteins. In a word, this work illustrated the regulatory function of KIF22/miR-122 axis in ESSC and provided potential targets for potential targets for ESSC treatment.

Keywords: Esophageal squamous cell carcinoma, KIF22, miR-122, proliferation, invasion

Introduction

Esophageal cancer (EC) ranks as the eighth most regularly analyzed cancer and the sixth most common cause of worldwide cancer-related death [1, 2]. EC consists of two histological sorts, esophageal squamous cell cancer (ESCC) and esophageal adenocarcinoma (EAC) [3]. In China, surpassing 100 cases of ESCC within 100,000 people happened per year. In spite of the great progress made in ESCC treatments, the 5-year survival rate of ESCC patients still persists at around 14% on account of metastasis [4]. Hence, illustrating the underlying mechanism of ESCC would give the knowledge to clarify the development of ESCC in China [5].

Kinesin superfamily (KIFs), facilitate various important functional molecules such as organelles, protein complexes, messenger RNAs (mRNAs) and chromosomes transported along the microtubules in cells during mitosis [6]. KIFs play important roles in various basic mechanisms of life, such as advanced brain functions such as memory and learning, neuron survival, and important developmental processes such as tumorigenesis [7, 8]. However, the role of most KIFs in tumorigenesis remains to a great extent obscure.

KIF22, a kinesin-like DNA-binding protein (Kid), is a member of the kinesin-10 family that regulates microtubule stability, synaptic development, and cytoskeleton dynamics [9-12]. KIF22 affinity for microtubules is under management of cyclin-dependent kinase 1 (CDK1)-mediated phosphorylation [13]. Although KIF22 plays a role as a motor protein during mitosis [14], it is in an exorbitant expression level in various types of carcinomas, such as mammary, cervical, ovarian and lung cancers, and is considered significant in tumorigenesis and cancer

progression. For example, the phosphorylation of KIF22 is a crucial element in the transcriptional regulation of the cell division cycle 25C (CDC25C) protein, leading to delayed exit of breast cancer mitosis [15]. Additionally, a recent research revealed that the internalization of lung cancer cells is delayed, and the EGFR signaling enhancement and coxsackie virus and adenovirus receptor (CAR) kinetic cell-cell junctions are regulated by KIF22-dependent microtubule dynamics [12]. It was also reported by Niu et al. that up-regulation of KIF22 was thought to have relations with tumor development and severe clinical outcome in prostate cancer patients [16]. However, the expression and potential mechanisms of KIF22 in ESCC need further exploration.

MicroRNA (miRNA), a type of non-coding RNA, has a length of 19-24 nucleotides [17], which can modulate cell proliferation, cell cycle, invasion and metastasis [18]. A great deal of findings have revealed that the exceptional expressions of microRNAs (miRNAs) are normally linked to the occurrence and development of tumors and have impacts on clinical effects of radiotherapy or chemotherapy [19]. Dysregulated miRNAs levels influence the development of cancer via regulating tumor-related pathways [20]. Therefore, exploring miRNAs function, especially related to the occurrence and development of ESCC, would inspire novel insights into its diagnosis and treatment target.

Herein, we showed that KIF22 was up-regulated in human ESCC tissues and cells compared with normal cell lines. KIF22 augmented cancer cell to proliferate, migrate and invade. KIF22 was also proved to regulated negatively by miR-122. miR-122 inhibitor significantly reversed the function of si-KIF22. Our discoveries provide a potential and promising clue for ESCC treatment.

Material and methods

Clinical samples

30 patients with ESCC had accepted routine surgical treatments at The Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University from May 2018 to November 2018, the tumor samples were extracted and hoarded using fluid nitrogen cryopreservation till RNA extraction. This research was granted by the Ethical Committee of The Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University, and meet the standard of the 1964 Helsinki Declaration. All patients included in this study signed informed consents.

Immunohistochemical staining

The specimens stored in formalin were embedded in paraffin, and then were spilt into 4 μm samples. Added 30 mL/L H_2O_2 to inactivate endogenous peroxidase and then blocked endogenous nonspecific with 10% goat serum. The slices were given with rabbit antihuman antibody to KIF22 at 4°C overnight. Biotin-labelled goat anti-rabbit antibody was added for further 30 min incubation at 37°C. Then the samples were stained with horseradish peroxidase-streptomycin, diaminobenzidine, and hematoxylin, in order. The results were recorded with 5 visual fields (20×) selected from each sample using Nikon Eclipse 80i.

Cell culture

HEEC (normal esophageal epithelial cell line) and 4 ESCC cell lines (TE1, TE10 ECa-109, Kyse-30, Kyse-70) were purchased from Fudan University Shanghai Cell Bank for this research, and was incubated with RPMI-1640 medium containing 10% FBS and 100 U/ml penicillin under 5% CO_2, at 37°C.

Cell transfection

The specific siRNAs against KIF22 (siKIF22), a scramble siRNA (siCtrl), KIF22 overexpression plasmid (pc and negative control plasmid were obtained from Sangon Biotech Co. Ltd. MiRNA vectors, including miR-122 inhibitors (miR-122 inhibitor) and miR-1222 inhibitor control clones (inhibitor NC) were gained from GenePharma (Guangzhou, China). Cells transfections were used with above vectors by Lipofectamine 2000 (Invitrogen) on the basis of the protocol.

Real-time quantitative reverse transcription-PCR (qRT-PCR) analysis

Cells or tissues were gathered and total RNA was extracted using Trizol reagent, KIF22 and miR-122 expression analyzed by qRT-PCR were performed as previously reported [21]. The

$2^{-\Delta\Delta Ct}$ method was used to calculate the content of KIF22 and miR-122 in human tissues and cultured cells [22]. The expression of KIF22 was positively correlated to β-actin, U6 was used as internal reference for miR-122. The primers are as follows: KIF22 forward 5'-CGGCCTTTTACCAATGAGAGC-3', reverse 5'-GACCAAGCAATTCTTTCTGAGACA-3'; β-actin forward 5'-CTCCATCCTGGCCTCGCTGT-3', reverse 5'-GCTGTCACCTTCACCGTTCC-3'; miR-122 forward 5'-TATTCGCACTGGATACGACACAAAC-3', reverse 5'-GCCCGTGGAGTGTGACAATGGT-3'; U6 forward 5'-GCTTCGGCAGCACATATACTAAAAT-3', U6 reverse 5'-CGCTTCACGAATTTGCGTGTCAT-3'.

Immunoblotting test

Protein isolation and immunoblotting test were excreted as previously reported. Antibodies against KIF22, p21, p27, cyclin D1, CDK2, E-cadherin, N-cadherin, Vimentin and β-actin (Abcam, Cambridge, UK) were used while β-actin was considered as the internal control.

Cell growth ability by MTT assay and BrdU incorporation assay

The cells were transferred to a 96-well plate with 5000 cells per well then incubated for 24 h, 48 h, 72 h and 96 h at 37°C, then incubated with 5 μg/mL MTT for another four hours. The measured absorbance at 450 nm through a microplate reader was employed to evaluate the growth ability. Bromodeoxyuridine (BrdU) incorporation assay was applied to estimate the capacity of proliferation. 10 μM BrdU (Thermo Fisher Scientific) was added and after 48 hours, colchicine (Sigma-Aldrich, USA) was added to dilute the concentration into 0.1 μM. Two days later, the collected cells were treated with Giemsa solution (Sigma-Aldrich, USA) for 10 minutes. Then the cells were photographed by a fluorescence microscope.

Transwell migration and invasion assay

For the migration assay, collected 200 μL of cell suspension to the Transwell upper chamber (Corning, Corning, NY, USA). In the upper chamber of each well, added 50 μL of serum-free medium. After 24 h culture, 4% paraformaldehyde was fixed for 30 min and 0.1% crystal violet was stained for 10 min. Recorded the image under the display lens and counted the number of migrating cells. In cell invasion experiment, added Matrigel to the Transwell upper chamber, and the following step was performed as the migration experiment after coagulation.

Apoptosis and cell cycle study

The cells were seeded to the 6-well plate (4×10^5 cells per well). After incubation, cells were collected and washed with phosphate buffer solution (PBS), resuspended in 500 μL binding buffer. 5 μL Annexin V-FITC solution and 5 μL PI were added to the buffer, then incubated for 15 min avoid light. The following analyze steps were performed on the flow cytometry machine.

Luciferase assay

The target wild-type KIF22 and mutant KIF22 were constructed and integrated into the pGL3 vector to construct pGL3-KIF22-wild type (KIF22-WT) and pGL3-KIF22-mutant (KIF22-MUT). After transfected with miR-122 mimics or negative control with pGL3-KIF22-WT or pGL3-KIF22-MUT reporter vector, respectively, using Lipofectamine 2000 under the manufacturer's instruction. Luciferase activity was determined after 24 h transfection.

Statistical analysis

Statistical data were arranged by SPSS 20.0. Independent sample t-test was for comparison between two groups, comparisons between multiple groups were analyzed by one-way ANOVA analysis. $P<0.05$ means significant difference statistically.

Results

KIF22 is in high expression level in ESCC tissues and cell lines

The expression of KIF22 in ESCC tissues and cell lines were measured firstly. KIF22 was highly upregulated in ESCC tissues both at mRNA and protein level (**Figure 1A** and **1B**) than in para-carcinoma normal tissues, that was also demonstrated by immunohistochemical analysis (**Figure 1C**). Compared with human esophageal epithelial cell line (HEEC), five ESCC cell lines (TE1, TE10, ECa-109, Kyse-30 and Kyse-170) exhibited significant upregulation of KIF22 examined through qRT-PCR and immunoblotting test (**Figure 1D** and **1E**). The results suggested a significant expression increase-

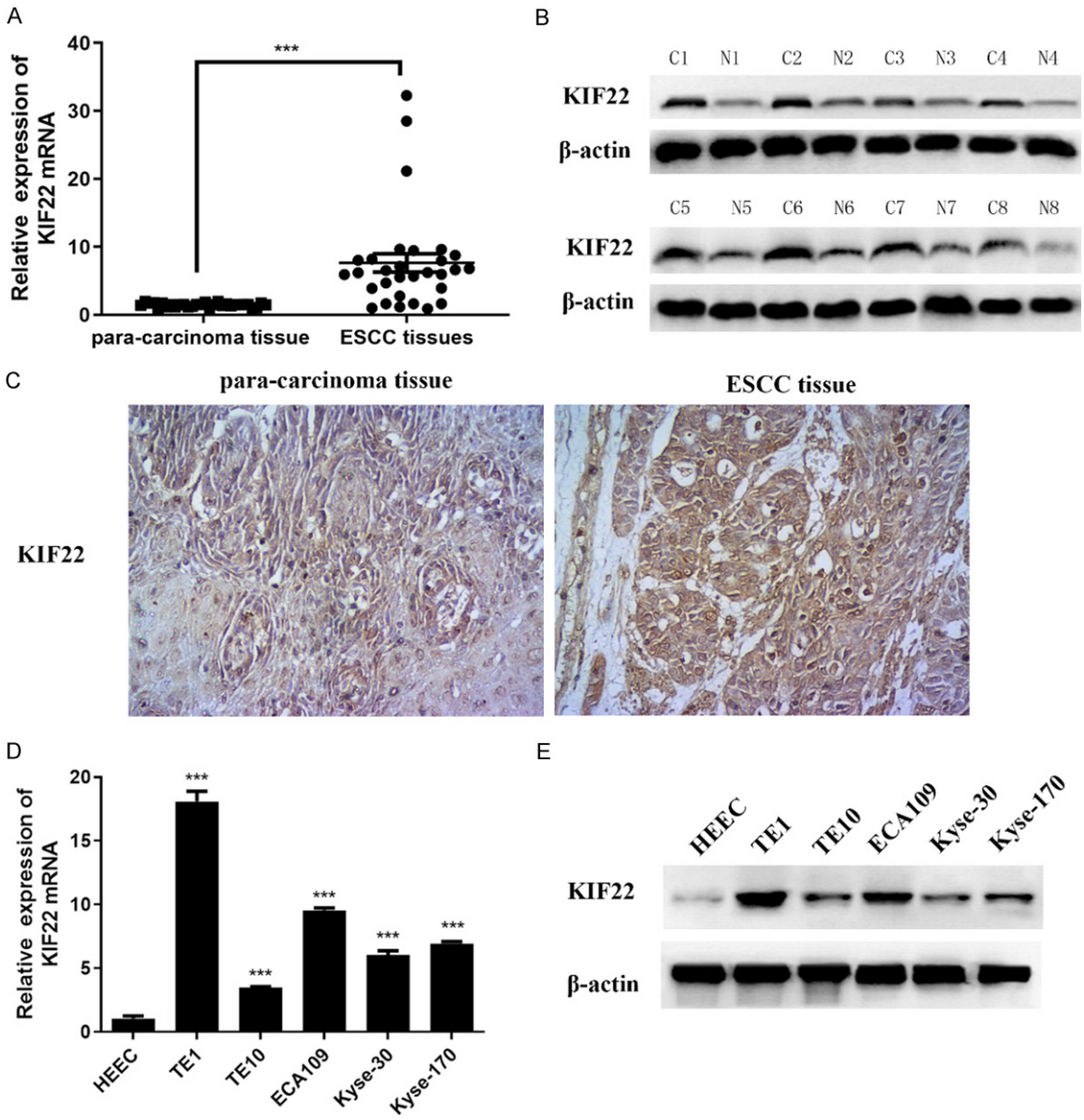

Figure 1. KIF22 is upregulated in ESCC tissues and cell lines. A, D. qRT-PCR was excreted to evaluate the level of KIF22 in ESCC tissues and cell lines. C. Immunohistochemistry of KIF22 in ESCC tissue and para-carcinoma tissue. B, E. The protein expression of KIF22 in ESCC tissues and cell lines was detected by western blot (C: ESCC cancer tissues; N: para-cancerous non-tumor tissues). **P*<0.05, ***P*<0.01, ****P*<0.001 vs. normal tissues or normal cells. Results were normalized with mean ± Standard deviation of 3 independent experiments provided.

ment of KIF22 in ESCC tissues and cell lines. Furthermore, the possible correlation between KIF22 expression level and the clinical features of ESCC patients were analyzed (**Table 1**). No remarkable relationship was observed between KIF22 expression levels and the age, gender, or tumor size of ESCC patients. Nevertheless, the KIF22 level was distinctly upregulated in ESCC patients with lower degree of differentiation, lymph node metastasis, and IIIa stage.

Knocking down KIF22 restrained ESCC cell capacity to proliferate, migrate and invade

TE-1 and ECa-109 cells were cultured to study the biological function and further exploration of KIF22 in ESCC. Specific siRNAs were used to silence the level of KIF22. Good efficiency of transfection was reached after 48 h by qRT-PCR (**Figure 2A**). Immunoblotting test was also carried out to verify the transfection efficiency

Table 1. KIF22 expression was associated with degree of differentiation, the presence of lymph node metastasis and TMN stage in ESCC

Clinical characteristics	Case (n)	Kif22 expression	*p*
Age (years)			0.95
<60	23	7.640±0.937	
≥60	28	7.613±0.864	
Gender			0.83
Male	39	7.526±1.125	
Female	12	7.594±1.026	
Tumor diameter (cm)			0.47
<4	34	7.341±0.824	
≥4	17	7.458±0.763	
Degree of differentiation			
Poor/undifferentiation	11	8.059±0.646	<0.01**
High/moderate undifferentiation	40	6.247±0.750	
Lymph node metastasis			<0.01**
Yes	13	7.945±0.827	
No	38	6.547±1.023	
TNM stage			<0.05*
I-II	36	6.863±0.744	
IIIa	15	7.926±0.815	

*P<0.05, **P<0.01.

results (**Figure 2B**), siKIF22#1 was selected for the following experiment. MTT and BrdU assays were conducted to evaluated cell viability. As shown in **Figure 2C** and **2D**. KIF22 knockdown significantly inhibited cell proliferation, whereas KIF22 overexpression exhibited the opposite effect.

Down-regulated KIF22 promoted ESCC cells migration, invasion, apoptosis and arrested cells in G0/G1 phase

Cell migration and invasion were performed with transwell assay. When siKIF22 was transfected, ESCC cells had significantly reduced migration and invasive capacity. These data together demonstrated that silencing KIF22 suppressed ESCC cells migration and invasion (**Figure 3A** and **3B**). Flow cytometric assay was applied to measure the quantity of cells in apoptosis and each cell cycle phase influenced by KIF22. As shown in **Figure 3C**, descending KIF22 level remarkably facilitated the apoptosis ratio of ESCC cells while increased KIF22 restrained cell apoptosis, compared to cells treated with siCtrl. Meanwhile, the results shown in **Figure 3D** demonstrated a significant increasement of G1 phase when KIF22 knockdown, compared with NC group, while that of G2 phase cells decreased significantly. These data elaborated that overexpression of KIF22 led to the distinct decrease of G1 phase and significantly arrested cells in S phase.

Knocking down KIF22 suppressed the expressions of cell cycle- and EMT-related proteins

The further mechanism of KIF22 on cell cycle and invasion was investigated. The cell cycle-related proteins including p21, p27, cyclin D1 and CDK2 were demonstrated as the straight or indirect downstream proteins of KIF22 by Western blot. Epithelial-mesenchymal transition (EMT) related proteins represent invasive capacity, as a result, EMT-related proteins were tested. When si-KIF22 was transfected in ESCC cells, p21 and p27 have high level while cyclin D1 and CDK2 have decreased expression (**Figure 4A**). Also, we proved the epithelial-mesenchymal transition (EMT) by measuring the levels of E-cadherin, N-cadherin and Vimentin. When si-KIF22 was transfected in ESCC cells, E-cadherin had high level while N-cadherin and Vimentin have decreased expression (**Figure 4B**).

miR-122 is of negative regulation function on KIF22

In order to explore the further mechanism of KIF22 suppressing the progression of ESSC cells, we identified miR-122 negatively regulated KIF22 in ESSC cells. Biological information prediction in miRanda pointed out that there were a series of KIF22 binding sites on the miR-122 sequence (**Figure 5A**). Subsequently, luciferase reporter assay was aimed to substantiate whether there was a direct interaction between WT KIF22 and miR-122. The results indicated that after co-transfection with the plasmid of pGL3-KIF22-wt and miR-122, the declining luciferase relative activity showed significant reduction than the cells co-transfected with KIF22 mutant-type. These evidences indicate that the expression of KIF22 in ESSC cells was negatively modulated through the direct

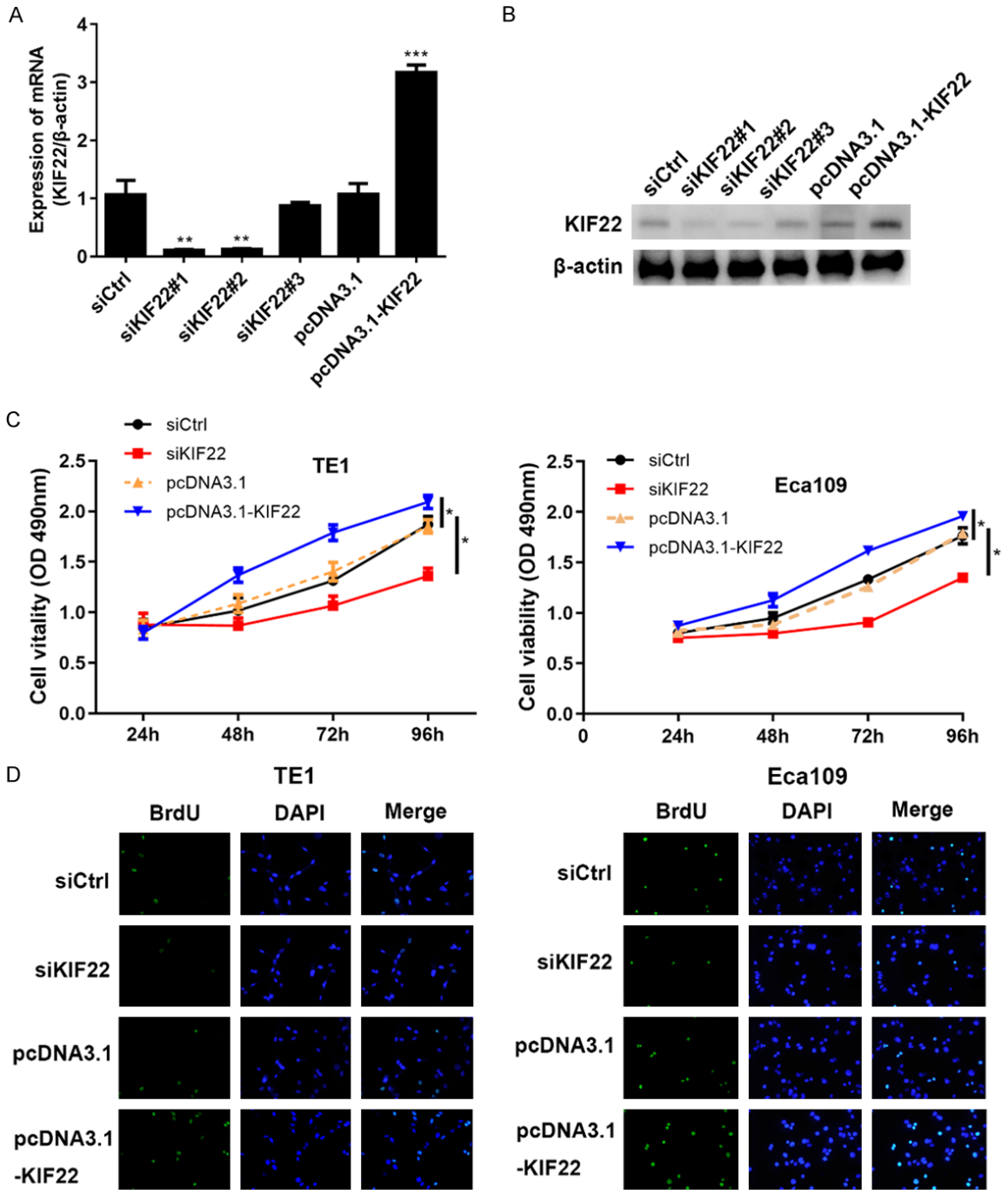

Figure 2. Knocking down KIF22 hindered ESCC cells to proliferate. (A) qRT-PCR was conducted to detect KIF22 mRNA level in ESCC cells after transfection. (B) Immunoblotting test was conducted to evaluate KIF22 expression in ESCC cells after transfection. Cell proliferation ability of ESCC after transfection by MTT (C) and BrdU (D) Assays. **P*<0.05, ***P*<0.01, ****P*<0.001 vs. respective controls. Results were normalized with mean ± Standard deviation of 3 independent experiments.

binging between miR-122 and KIF22 (**Figure 5B**).

Next, western blot and qRT-PCR were adapted to verifiy the influence of miR-122 overexpression on KIF22, explore the role of miR-122 in regulating endogenous KIF22 expression. The results suggested that miR-122 overexpression reduced KIF22 mRNA level (**Figure 5C**) and protein level (**Figure 5D**) in ESSC cells.

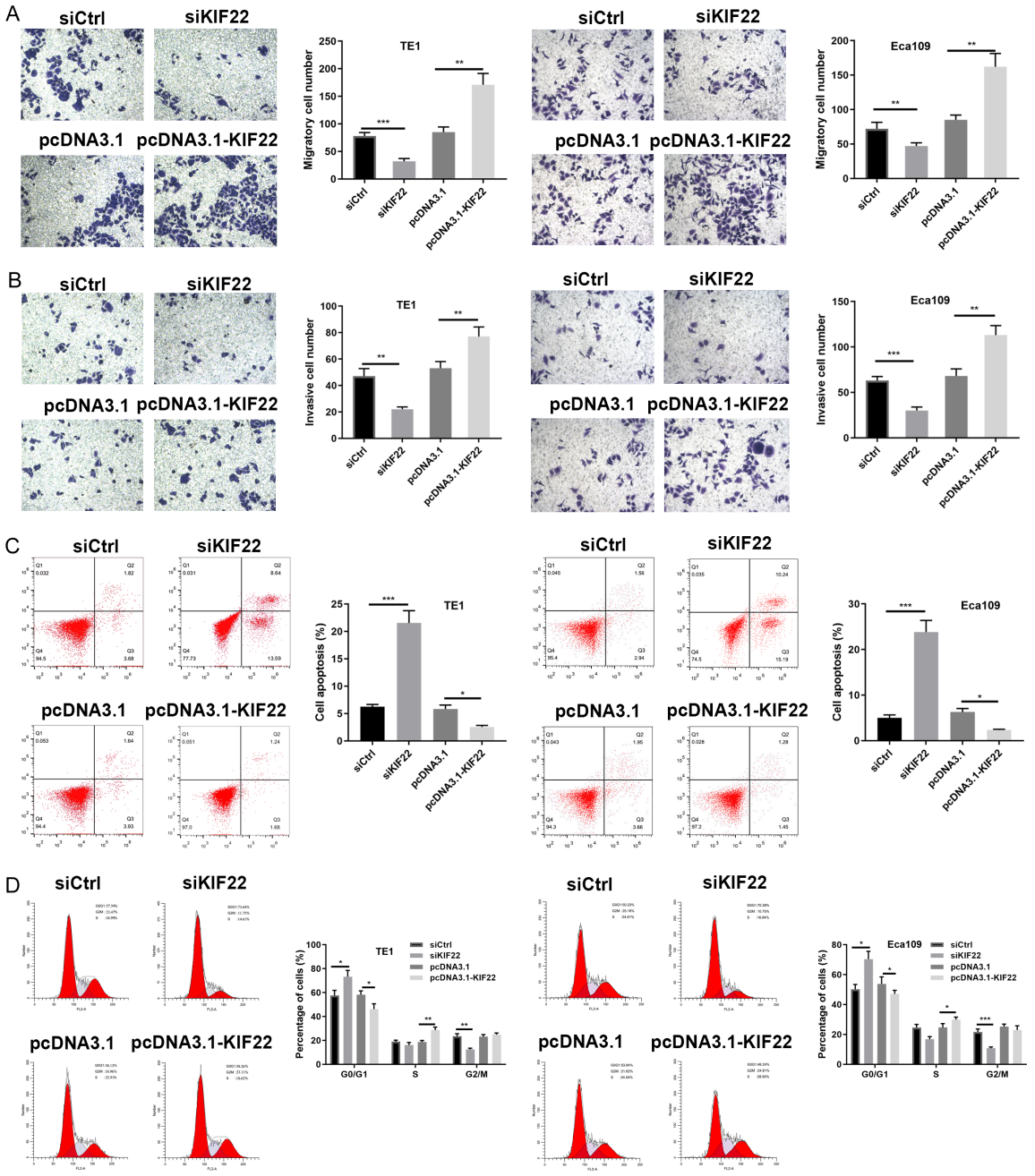

Figure 3. Down-regulation of KIF22 promoted ESCC cells migration, invasion, apoptosis and arrested cells in G0/G1 phase. The effect of KIF22 knockdown on migration (A) and invasion (B) of ESCC cells was assessed using Transwell assay. (C) Flow cytometric analysis of KIF22 on cell apoptosis. (D) Flow cytometry was employed to detect the function of KIF22 on the cell cycle of ESCC. **P*<0.05, ***P*<0.01, ****P*<0.001 vs. respective controls. Results were normalized with mean ± Standard deviation of 3 independent experiments.

Inhibition of miR-122 altered the function of KIF22 knockdown

After transfection, miR-122 inhibitor significantly impaired the anti-proliferative effect of KIF22 knockdown assessed by the MTT and BrdU tests (**Figure 6A** and **6B**). Furthermore, ESCC cells have increased migration and invasive capacity when transfected miR-122 inhibitor (**Figure 6C** and **6D**). These data together dem-

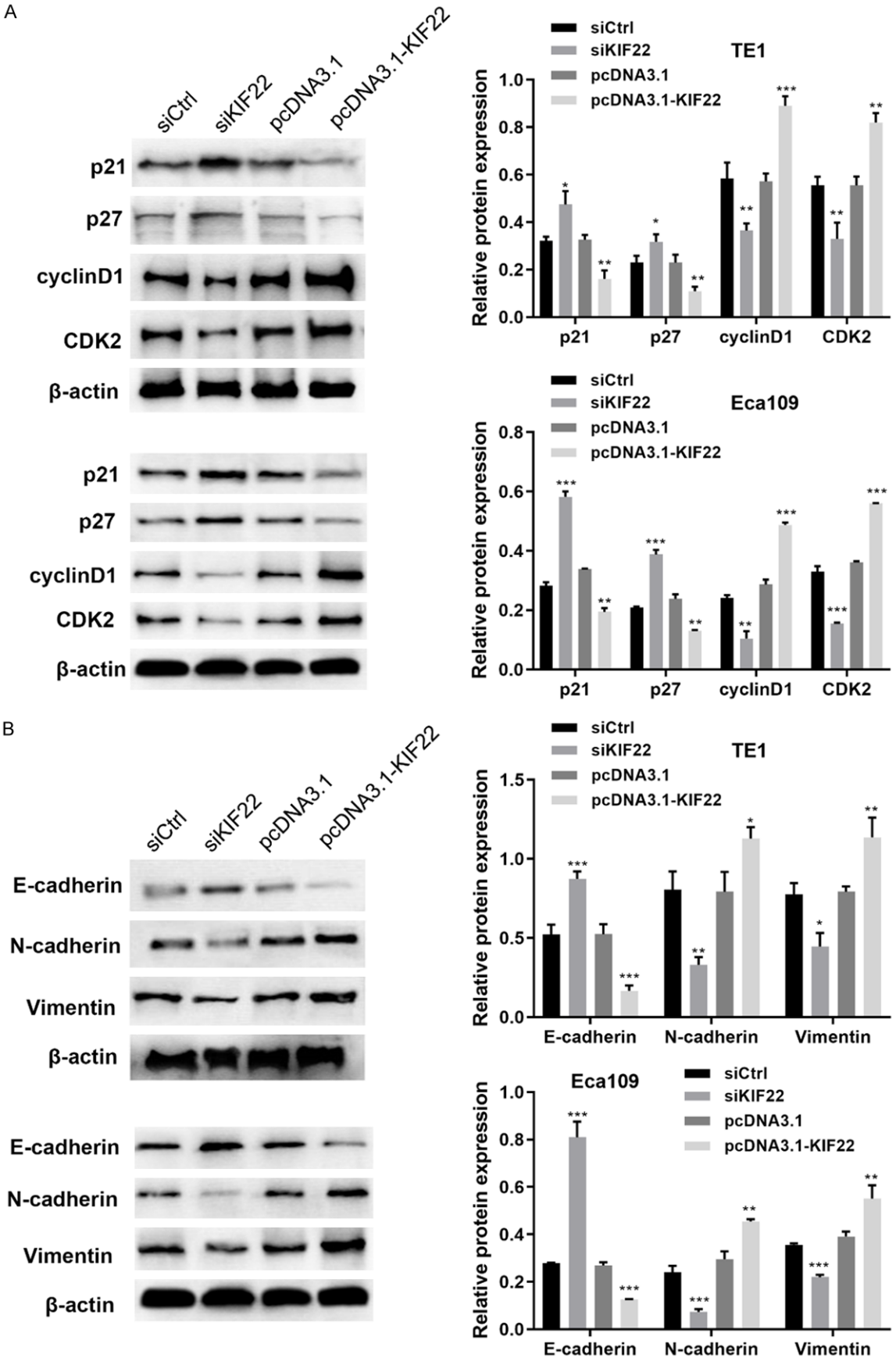
A
siCtrl
siKIF22
pcDNA3.1
pcDNA3.1-KIF22
p21
p27
cyclinD1
CDK2
β-actin
TE1
Eca109
Relative protein expression
B
E-cadherin
N-cadherin
Vimentin

Figure 4. KIF22 regulated the process of ESSC by regulating cell cycle- and EMT-related proteins. A. Expression of cell cycle-related proteins after transfection. B. Expression levels of EMT-related proteins. Results were normalized with mean ± Standard deviation of 3 independent experiments. **P*<0.05, ***P*<0.01, ****P*<0.001 vs. respective controls. Results were normalized with mean ± Standard deviation of 3 independent experiments.

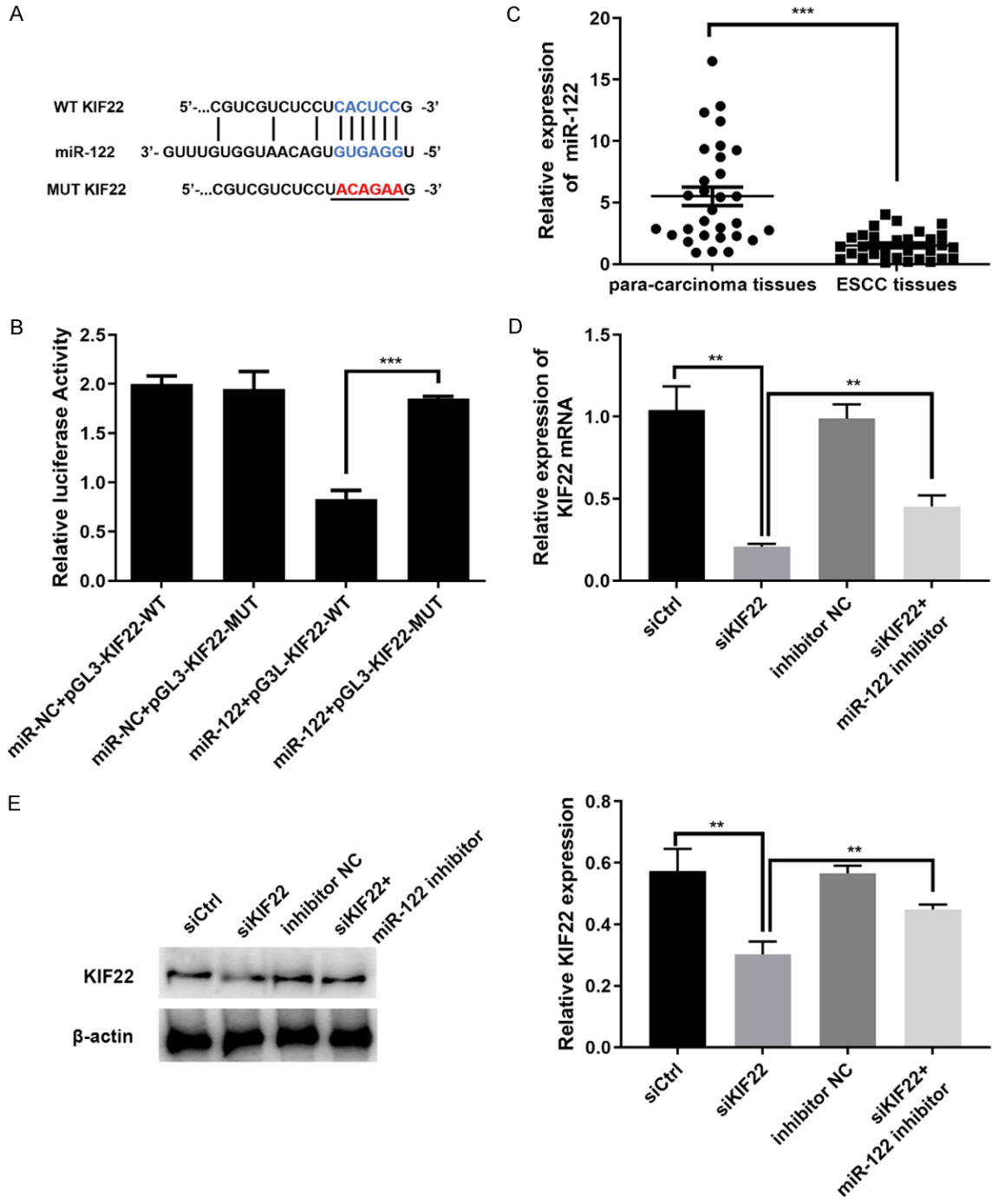

Figure 5. KIF22 is negatively regulated by miR-122. (A) Predicted binding sites of miR-122 in the KIF22. (B) Relative luciferase activities in ESSC cells with different co-transfections. (C) The expression of miR-122 in ESSC tissues and para-carcinoma tissues. The expression of KIF22 mRNA (D) and protein (E) in ESSC cells after transfections. **P*<0.05, ***P*<0.01, ****P*<0.001 vs. respective controls. Results were normalized with mean ± Standard deviation of 3 independent experiments.

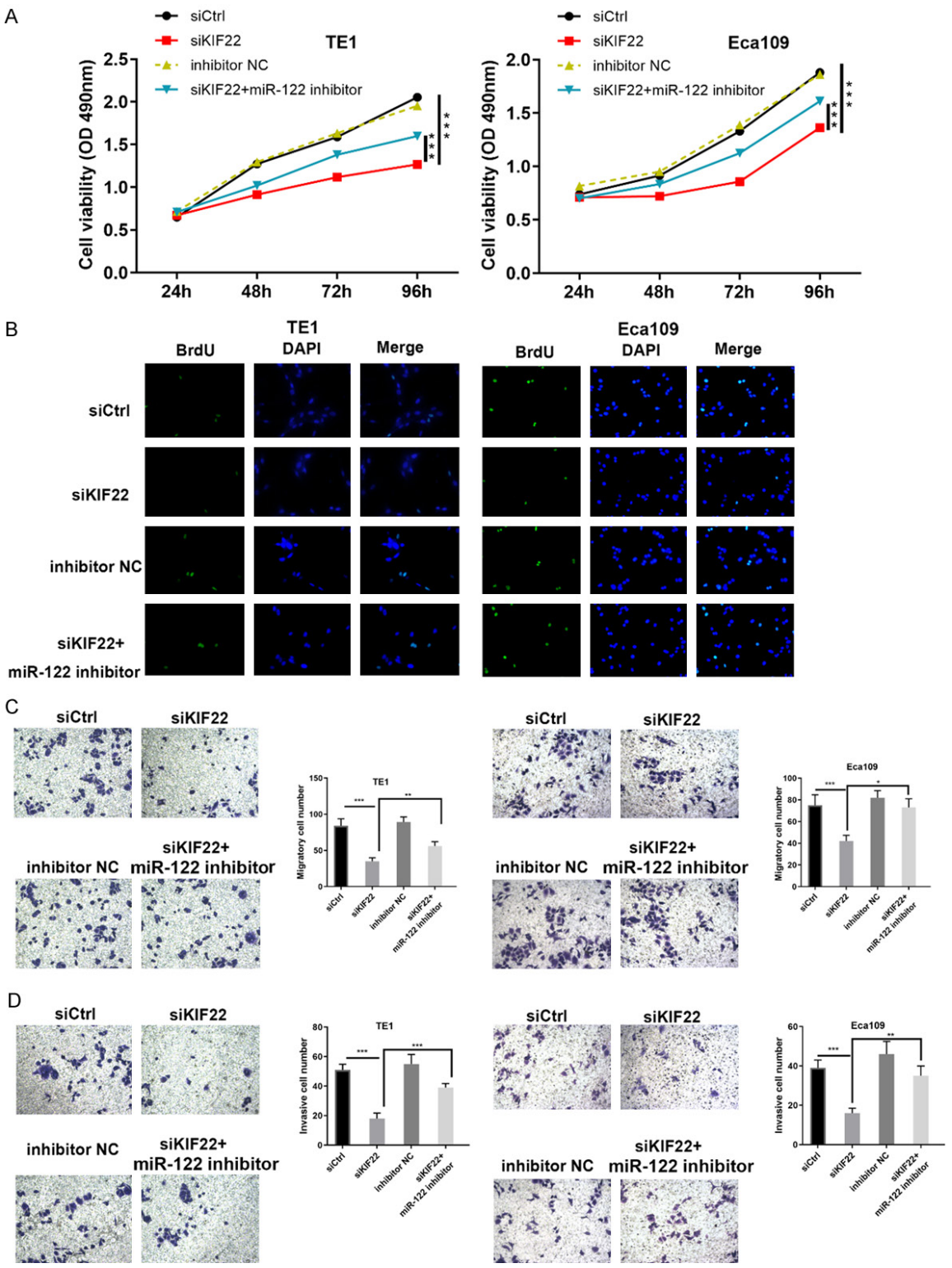

Figure 6. Down-regulation of miR-122 reversed the function of KIF22 knockdown. MTT assay (A) and BrdU assay (B) were conducted to explore miR-122 inhibitor effect on proliferation. The effect of miR-122 inhibitor on migration (C) and invasion (D) of ESCC cells was assessed using Transwell assay. **P*<0.05, ***P*<0.01, ****P*<0.001 vs. siKIF22 group. Results were normalized with mean ± Standard deviation of 3 independent experiments.

onstrated that down-regulation of miR-122 reversed the function caused by KIF22 knockdown in ESCC.

Down-regulation of miR-122 level reversed the effect on cell cycle- and EMT-related proteins brought by KIF22 knockdown

Immunoblotting test was used to quantify the relative protein level of p21, p27, cyclin D1 and CDK2. The results revealed that down-regulation of miR-122 may decrease the upregulated expression of p21, p27 caused by siKIF22, while cyclin D1 and CDK2 levels were markedly increased (**Figure 7A**).

Also, the EMT-related proteins were affected by miR-122 inhibitor. Depicted in **Figure 7B**, miR-122 inhibitor group significantly impaired E-cadherin level which is upregulated by siKIF22, whereas increased the quantity of N-cadherin and Vimentin when compared to the siKIF22 group (**Figure 7B**).

Discussion

ESCC is predominant among EC in China. It was urgent to investigate the specific mechanism for ESCC treatment. So far, over 45 individuals of the KIF members have been distinguished in mammalian cells, and a few are identified as promising biomarkers and targets for cancer therapy [23].

KIF22 is a kind of positive microtubule kinesin, which is essential for mitosis in cells. Its affinity with microtubules is regulated by the CDK1-mediated phosphorylation process. The affinity of microtubules is reduced, but its affinity for chromosomes is enhanced [24]. Previous literatures have demonstrated that in human colorectal cancer tissues, the increased KIF22 expression has high correlation with tumor stage and clinical stage. Down-regulation of KIF22 hindered cell proliferation and the growth of xenograft [25].

The present data firstly demonstrated that the expression of KIF22 was remarkably increased in ESCC tissues and cell lines. Besides, KIF22 level was obviously ascending in ESCC patients with lower degree of differentiation, lymph node metastasis, and IIIa stage. In order to further understand its related functions, si-KIF22 was transfected in this study to evaluate the capacity to proliferate, migrate and invade in TE1 and Eca109 cells. KIF22 knockdown suppressed ESCC cell proliferation by MTT and BrdU assays, indicating that KIF22 took part in the progress of ESCC cells and providing a promising candidate target for ESCC [9, 26].

Downregulation of KIF22 obviously suppressed the migratory and invasive ability, accelerated the ESCC cells apoptosis. It was also found that inhibiting KIF22 expression can regulate the cell cycle, leading to a higher proportion of cells in G0/G1 phase.

Several researches have revealed in context of KIF22 mediated tumor regulation, that KIF22 could regulate microtubule dynamics to delay EGFR internalization and enhance EGFR signaling in lung tumor growth [12]. KIF22 promoted gastric cells growth and migration abilities via MAPK-ERK pathways [27]. The specific mechanism of KIF22 on ESCC still need more investigations. Cell proliferation is closely determined by the regulation of cell cycle progression. p21, p27, cyclin D1 and CDK 2 are key links in cell proliferation [28]. In this study these proteins level were determined by western blotting, indicating that G1/S phase transition induced by KIF22 was related to knockdown of cyclin-D1 and CDK2 and upregulation of p21 and p27. As a result, KIF22 was necessary in regulating cell cycle progression.

EMT pathway is closely related to tumor metastasis. The results of a cascade of multiple extracellular signaling molecules that activate downstream proteins upon EMT [29]. The role of KIF22 on the effect of EMT-related proteins revealed that higher-expressed KIF22 promoted EMT, so as to aggravate ESCC invasion and metastasis.

Recently, miRNAs were found as key factors in tumorigenesis and metastasis. Bioinformatics prediction and luciferase reporter assay proved that KIF22 is a target gene of miR-122, that was reported that deregulated in diverse cancers including hepatobiliary cancer, non-small-cell lung cancer and gastric carcinoma [30]. Besides, miR-122 enhances the suppressive effect of ionizing radiation, IR, on cancer cell anchors [31]. The level of miR-122 is related to the development, progress and prognosis of hepatocellular carcinoma (HCC) [32]. It is evident that miR-122 can regulate HCC cells

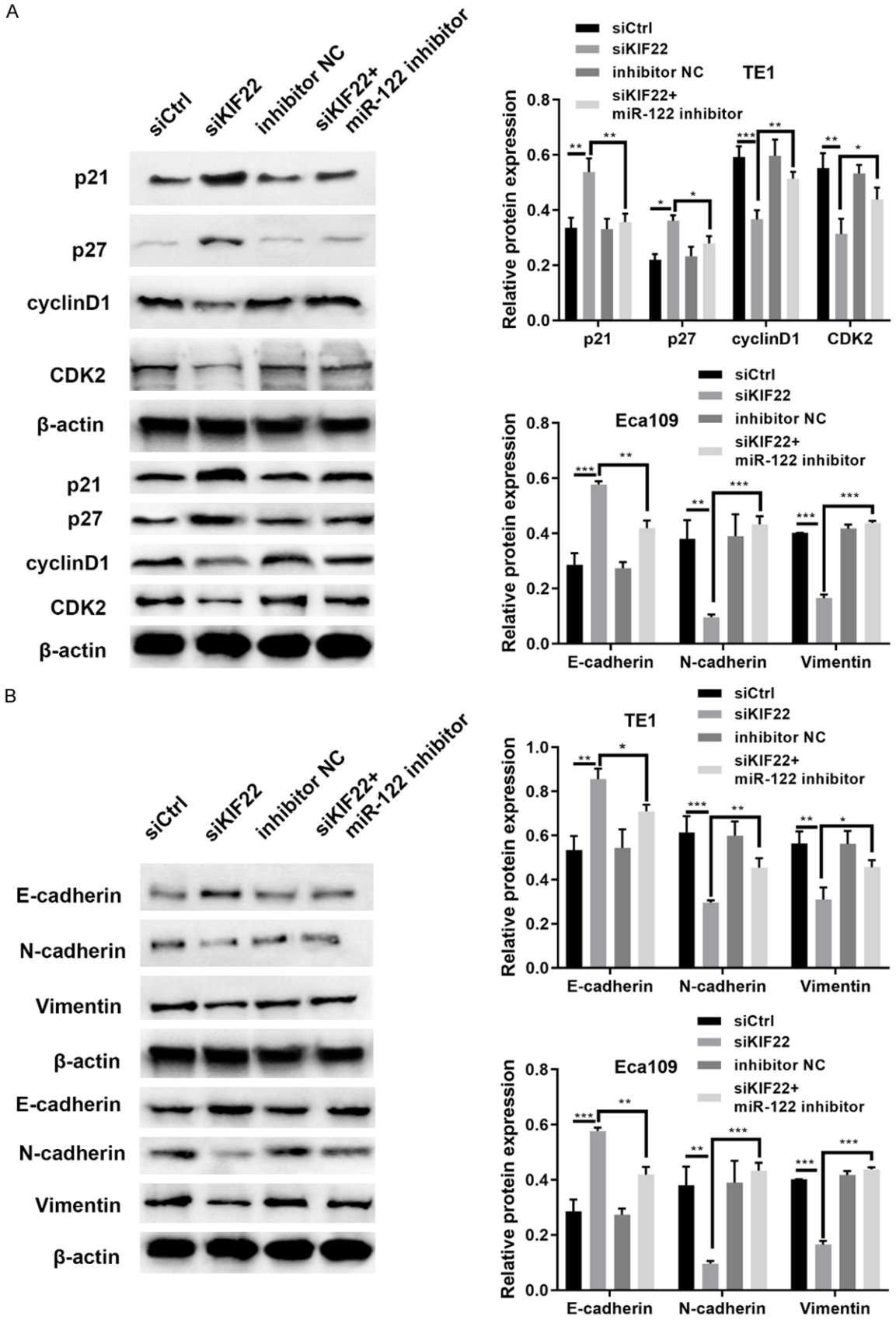
A
siCtrl
siKIF22
inhibitor NC
siKIF22+ miR-122 inhibitor
p21
p27
cyclinD1
CDK2
β-actin
TE1
Eca109
Relative protein expression
E-cadherin
N-cadherin
Vimentin
B

Figure 7. Down-regulation of miR-122 reversed the function of low-expressed KIF22 on cell cycle- and EMT-related proteins. A. Cell cycle-related proteins levels after transfection. B. Western blot was aimed to confirmed EMT-related proteins levels. **P*<0.05, ***P*<0.01, ****P*<0.001 vs. siKIF22 group. Results were normalized with mean ± Standard deviation of 3 independent experiments.

chemical sensitivity [33]. In addition, high levels of miR-122 are closely related to metastasis in breast cancer patients, while miR-122, which secretes cancer cells, promotes metastasis by increasing the availability of nutrients in the pre-metastatic niche [34]. miR-122 was also proved that inhibited breast cancer cell growth and tumorigenesis by targeting IGF1R, and suppressed metastasis and EMT of non-small-cell lung cancer cells [28, 35]. These previous work concluded that miR-122 may work as a regulatory gene in various sorts of cancer, but the expression and role of miR-122 and how miR-122/KIF22 axis works in ESCC remain needs in-depth studies.

Given that KIF22 and miR-122 are co-factors that potentially affect ESCC, we added si-KIF22 and miR-122 inhibitor to the stably cultured ESCC cells. After transfection with corresponding plasmids, miR-122 inhibitor significantly impaired the anti-proliferative effect of KIF22 knockdown determined by the MTT and BrdU assays. Furthermore, the expressions of cell cycle- and EMT-related proteins regulated by KIF22 were also negatively mediated by miR-122. These findings indicate that miR-122 and KIF22 interactions can be evaluated as a new strategy for the future in controlling the growth and metastasis of ESSC cells.

Our current data indicated that the descending level of miR-122 inhibited KIF22-mediated ESCC cell progress, suggesting that KIF22/miR-122 axis may be a potential biomarker for ESCC.

Nevertheless, there are still many confusions about the function of the KIF22/miR-122 axis, such as whether the KIF22/miR-122 axis has other interactions to promote ESCC progression or whether this axis works on in vivo. After further explorations, the results will assist us understand in-depth function of KIF22/miR-122 axis in ESCC and other tumors.

In conclusion, it was elucidated that KIF22 is a uniq direct target of miR-122, and KIF22/miR-122 axis is significant for ESCC progression. The results indicated that therapeutic strategies targeting KIF22/miR-122 axis can be potential and promising for the ESCC treatment.

Acknowledgements

We thanked the support from National Natural Science Foundation of China (11705095, 82073339); Young Talent Development Plan of Changzhou Health Commission (CZQM2020074); Changzhou Sci & Tech Program (CJ20200053); 333 High-level personnel training Project (BRA2019025); The Key Research & Development Program of Jiangsu Province (BE2020637); Wuxi double hundred young and middle-aged medical and health top-notch talent project (No. 202014).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jian-Lin Wang, Department of Radiotherapy, The Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University, No. 29, Xinglong Lane, Tianning District, Changzhou 213003, Jiangsu Province, China. Tel: +86-519-88118723; E-mail: wangjianlin@njmu.edu.cn

References

[1] Zhang Y. Epidemiology of esophageal cancer. World J Gastroenterol 2013; 19: 5598-5606.
[2] Conteduca V, Sansonno D, Ingravallo G, Marangi S, Russi S, Lauletta G and Dammacco F. Barrett's esophagus and esophageal cancer: an overview. Int J Oncol 2012; 41: 414-424.
[3] Zhang F, Yang Z, Cao M, Xu Y, Li J, Chen X, Gao Z, Xin J, Zhou S, Zhou Z, Yang Y, Sheng W and Zeng Y. MiR-203 suppresses tumor growth and invasion and down-regulates MiR-21 expression through repressing Ran in esophageal cancer. Cancer Lett 2014; 342: 121-129.
[4] Koshy M, Esiashvilli N, Landry JC, Thomas CR and Matthews RH. Multiple management modalities in esophageal cancer: combined modality management approaches. Oncologist 2004; 9: 147-159.
[5] Wang M, Li Y, Yang Y, Liu X, Zang M, Li Y, Yang K, Yang W and Zhang S. Long non-coding RNA DLX6-AS1 is associated with malignant pro-

gression and promotes proliferation and invasion in esophageal squamous cell carcinoma. Mol Med Report 2019; 19: 1942-1950.
[6] Hirokawa N and Noda Y. Intracellular transport and kinesin superfamily proteins, KIFs: structure, function, and dynamics. Physiol Rev 2008; 88: 1089-1118.
[7] Miki H, Okada Y and Hirokawa N. Analysis of the kinesin superfamily: insights into structure and function. Trends Cell Biol 2005; 15: 467-476.
[8] Hirokawa N, Noda Y, Tanaka Y and Niwa S. Kinesin superfamily motor proteins and intracellular transport. Nat Rev Mol Cell Biol 2009; 10: 682-696.
[9] Yu Y and Feng YM. The role of kinesin family proteins in tumorigenesis and progression: potential biomarkers and molecular targets for cancer therapy. Cancer 2010; 116: 5150-5160.
[10] Miki H, Okada Y and Hirokawa N. Analysis of the kinesin superfamily: insights into structure and function. Trends Cell Biol 2005; 15: 467-476.
[11] Park SM, Littleton JT, Park HR and Lee JH. Drosophila homolog of human KIF22 at the autism-linked 16p11. 2 loci influences synaptic connectivity at larval neuromuscular junctions. Exp Neurobiol 2016; 25: 33-39.
[12] Pike R, Ortiz-Zapater E, Lumicisi B, Santis G and Parsons M. KIF22 coordinates CAR and EGFR dynamics to promote cancer cell proliferation. Sci Signal 2018; 11: eaaq1060.
[13] Ohsugi M, Tokai-Nishizumi N, Shiroguchi K, Toyoshima YY, Inoue J and Yamamoto T. Cdc2-mediated phosphorylation of Kid controls its distribution to spindle and chromosomes. EMBO J 2003; 22: 2091-2103.
[14] Tokai N, Fujimoto-Nishiyama A, Toyoshima Y, Yonemura S, Tsukita S, Inoue J and Yamamota T. Kid, a novel kinesin-like DNA binding protein, is localized to chromosomes and the mitotic spindle. EMBO J 1996; 15: 457-467.
[15] Yu Y, Wang XY, Sun L, Wang YL, Wan YF, Li XQ and Feng YM. Inhibition of KIF22 suppresses cancer cell proliferation by delaying mitotic exit through upregulating CDC25C expression. Carcinogenesis 2014; 35: 1416-1425.
[16] Zhang Z, Xie H, Zhu S, Chen X, Yu J, Shen T, Li X, Shang Z and Niu Y. High expression of KIF22/kinesin-like DNA binding protein (Kid) as a poor prognostic factor in prostate cancer patients. Med Sci Monit 2018; 24: 8190-8197.
[17] Shukla GC, Singh J and Barik S. MicroRNAs: processing, maturation, target recognition and regulatory functions. Mol Cell Pharmacol 2011; 3: 83-92.
[18] Zhang J, Xu Y, Liu H and Pan Z. MicroRNAs in ovarian follicular atresia and granulosa cell apoptosis. Reprod Biol Endocrinol 2019; 17: 1-11.
[19] Wang C and Qiao C. MicroRNA-190b confers radio-sensitivity through negative regulation of Bcl-2 in gastric cancer cells. Biotechnol Lett 2017; 39: 485-490.
[20] Reddy KB. MicroRNA (miRNA) in cancer. Cancer Cell Int 2015; 15: 38.
[21] Zhang K, Chen J, Song H and Chen LB. SNHG16/miR-140-5p axis promotes esophagus cancer cell proliferation, migration and EMT formation through regulating ZEB1. Oncotarget 2018; 9: 1028.
[22] Ginzinger DG. Gene quantification using real-time quantitative PCR: an emerging technology hits the mainstream. Exp Hematol 2002; 30: 503-512.
[23] Yu Y and Feng YM. The role of kinesin family proteins in tumorigenesis and progression: potential biomarkers and molecular targets for cancer therapy. Cancer 2010; 116: 5150-5160.
[24] Ohsugi M, Tokai-Nishizumi N, Shiroguchi K, Toyoshima YY, Inoue Ji and Yamamoto T. Cdc2-mediated phosphorylation of Kid controls its distribution to spindle and chromosomes. EMBO J 2003; 22: 2091-2103.
[25] Li B, Zhu FC, Yu SX, Liu SJ and Li BY. Suppression of KIF22 inhibits cell proliferation and xenograft tumor growth in colon cancer. Cancer Biother Radiopharm 2020; 35: 50-57.
[26] Hirokawa N, Noda Y, Tanaka Y and Niwa S. Kinesin superfamily motor proteins and intracellular transport. Nat Rev Mol Cell Biol 2009; 10: 682-696.
[27] Yu ZY, Jiang XY, Zhao RR, Qin JJ, Luo CJ, Ren YX, Ren W, Ma ZJ and Jiao ZY. Effect of KIF22 on promoting proliferation and migration of gastric cancer cells via MAPK-ERK pathways. Chin Med J 2020; 133: 919.
[28] Qin H, Sha J, Jiang C, Gao X, Qu L, Yan H, Xu T, Jiang Q and Gao H. mir-122 inhibits metastasis and epithelial-mesenchymal transition of non-small-cell lung cancer cells. Onco Targets Ther 2015; 8: 3175.
[29] Brabletz T, Kalluri R, Nieto MA and Weinberg RA. EMT in cancer. Nat Rev Cancer 2018; 18: 128.
[30] Qiu Y, Yang J, Bian S, Chen G and Yu J. PPARγ suppresses the proliferation of cardiac myxoma cells through downregulation of MEF2D in a miR-122-dependent manner. Biochem Biophys Res Commun 2016; 474: 560-565.
[31] Zhang HS, Zhang FJ, Li H, Liu Y, Du GY and Huang YH. Tanshinone IIA inhibits human esophageal cancer cell growth through miR-122-mediated PKM2 down-regulation. Arch Biochem Biophys 2016; 598: 50-56.

[32] Bandiera S, Pfeffer S, Baumert TF and Zeisel MB. miR-122-a key factor and therapeutic target in liver disease. J Hepatol 2015; 62: 448-457.
[33] Basu S and Bhattacharyya SN. Insulin-like growth factor-1 prevents miR-122 production in neighbouring cells to curtail its intercellular transfer to ensure proliferation of human hepatoma cells. Nucleic Acids Res 2014; 42: 7170-7185.
[34] Venkatadri R, Muni T, Iyer A, Yakisich J and Azad N. Role of apoptosis-related miRNAs in resveratrol-induced breast cancer cell death. Cell Death Dis 2016; 7: e2104-e2104.
[35] Wang B, Wang H and Yang Z. MiR-122 inhibits cell proliferation and tumorigenesis of breast cancer by targeting IGF1R. PLoS One 2012; 7: e47053.