MicroRNA-1271-5p inhibits the tumorigenesis of ovarian cancer through targeting E2F5 and negatively regulates the mTOR signaling pathway

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Research

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Abstract

Background: MicroRNA-1271-5p (miR-1271-5p) has been reported to participate in the progression of many human cancers. However, the role of miR-1271-5p still remains unclear in ovarian cancer (OC). Therefore, we explored the effect of miR-1271-5p on the development of OC in present study.

Methods: We measured the miR-1271-5p expression via the qRT-PCR assay. Then the function of miR-1271-5p was analyzed through MTT and Transwell assays. The relationship among miR-1271-5p and E2F5 was verified by dual luciferase assay. The protein expression levels were examined through western blot.

Results: MiR-1271-5p was downregulated in OC tissues which predicted poor prognosis of OC patients. Moreover, E2F5 was a direct target of miR-1271-5p in OC. And miR-1271-5p suppressed cell proliferation, migration and invasion in OC through targeting E2F5. Furthermore, E2F5 was upregulated in OC tissues which predicted poor prognosis of OC patients. Besides that, miR-1271-5p suppressed EMT and mTOR pathway in OC.

Conclusion: MiR-1271-5p inhibited the tumorigenesis of OC through targeting E2F5 and negatively regulated the mTOR signaling pathway.

Introduction

Ovarian cancer (OC) is one of the most common cancers in the female reproductive organs, ranking third only to cervical cancer and uterine cancer [1]. But the death caused by OC is the first in all kinds of gynecological tumors, posing a serious threat to women's life [2]. At present, the etiology of OC is unclear, which may be related to age, fertility, blood type, mental factors and environment [3]. Moreover, the treatment of OC is different due to different pathological types, and operation combined with chemotherapy is usually used to treat OC [4, 5]. In addition, the five-year survival rate of OC patients is very low, only 25–30% [6]. However, if it is found early, 90% of the OC patients will survive; Later, when cancer cells spread to the ovary, the survival rate is less than 30% [7]. Therefore, early detection and early treatment are very important to improve the survival rate of OC patients.

In recent years, microRNAs (miRNAs) have been paid more and more attention because of their specific function in various cancers and disease. Attention is mainly focused on their inhibitory effect on the expression of targeted genes through binding with its 3'-UTR [8]. Moreover, many miRNAs have been reported to regulate the progress of OC. For example, miR-365 inhibited OC progression by targeting Wnt5a [9]. Qu et al. reported that miR-1 inhibited cell proliferation and migration in OC through c-Met pathway [10]. Inversely, miR-205 was found to promote cell invasion by repressing TCF21 in human OC [11]. Liu et al. demonstrated that miR-216a promoted the metastasis and EMT of OC by suppressing the PTEN/AKT pathway [12]. These studies indicated that miRNA could function as a biomarker and target in OC. Especially, miR-1271 has been found to express abnormally and show different functions in human cancers. Liang et al. found that circ-ABCB10 promoted breast cancer proliferation and progression

through sponging miR-1271 [13]. Moreover, miR-1271 had been found to function as a metastasis and EMT inhibitor in human HCC by targeting the PTP4A1/c-Src axis [14]. And suppression of Capn4 by miR-1271 was found to impede the proliferation and invasion of colorectal cancer cells [15]. In addition, expression and role of miR-1271 was reported to affect the pathogenesis of osteosarcoma [16]. However, it was reported that miR-1271 promoted non-small-cell lung cancer cell proliferation and invasion via targeting HOXA5 [17]. Previous studies suggested that miR-1271 displayed different roles in different human cancers. And these studies have stimulated our desire to explore the role of miR-1271 in OC.

As a member of E2F family, downregulation of E2F transcription factor 5 (E2F5) had been found to inhibit cell proliferation in hepatocellular carcinoma cells [18]. Moreover, upregulation of E2F5 had been identified in many human cancers, such as gastric cancer [19], breast cancer [20] and glioblastoma [21]. Furthermore, miR-98 had been found to delay skeletal muscle differentiation by downregulating E2F5 [22]. And E2F5 had been identified as an independent prognostic factor in esophageal squamous cell carcinoma [23]. However, the specific function of E2F5 in OC still remains unclear and need to be explored.

The present study investigated the abnormal expression of miR-1271-5p in OC which could predict the prognosis of OC. And the functions of miR-1271-5p were also analyzed for the biology activities associated with the development of OC. The relationship between miR-1271-5p and E2F5 was also confirmed in OC. In addition, the effect of miR-1271-5p on EMT and mTOR signaling pathway was analyzed as well. These findings would contribute to better understanding the pathogenesis of OC.

Materials And Methods

Clinical tissues

Forty-five human OC tissues and adjacent normal ovary tissues were acquired from The Third Affiliated Hospital of Soochow University (The First People's Hospital of Changzhou) after receiving signature written informed consent. All OC patients received no treatment prior to the operation. Then theses tissues were frozen in liquid nitrogen and then stored in the − 80℃ refrigerator to be used in the further experiment. This experiment was approved by the Institutional Ethics Committee of The Third Affiliated Hospital of Soochow University (The First People's Hospital of Changzhou).

Cell Lines Culture

The A2780, SKOV3, OVCAR cell lines and human normal ovarian epithelial cell line IOSE80 were used for this experiment. These cell lines were obtained from Tumor Cell Bank of the Chinese Academy of Medical Science (Beijing, China). Then these cell lines were seeded in RPMI-1640 medium with 10% fetal bovine serum (FBS) and cultured at 37 °C with 5% CO_2.

Cell Transfection

The miR-1271-5p mimic or inhibitor, miR-1271-5p plasmid and negative control (NC) were obtained from Ribobio (Guangzhou, China). Then they were transferred into SKOV3 cells with Lipofectamine 2000 (Invitrogen, Carlsbad, USA) based on the manufactures' protocols.

Quantitative Rt-pcr

TRIzol reagent (Invitrogen, Carlsbad, USA) was applied for extracting total RNA according to the standard method. And the synthesis of cDNA was conducted by using PrimeScript RT reagent kit (TaKaRa, USA). Quantitative RT-PCR was carried out through the SYBR Premix Ex Taq (TaKaRa, USA) on ABI 7500 Fast system (Applied Biosystems, CA, USA). U6 or GAPDH was used as control for miR-1271-5p or E2F5. And their expressions were calculated using the $2^{-\triangle\triangle ct}$ method. The forward primer for miR-1271-5p was 5'-CTT GGC ACC TAG CAA GCA CTC A-3', and the reverse primer was 5'-TAT GGT TGT TCT CCT CTC TGT CTC-3'. The internal control for miR-1271-5p was GAPDH (forward, 5'-CGG AGT CAA CGG ATT TGG TCG TAT-3'; reverse, 5'-AGC CTT CTC CAT GGT GGT GAA GAC-3'). The primers for E2F5 were 5'-CCT GTT CCC CCA CCT GAT G-3 (forward) and 5'-TTT CTG TGG AGT CAC TGG AGT CA-3' (reverse). The internal control was U6 (forward, 5'-CTC GCT TCG GCA GCA CA-3'; reverse, 5'-AAC GCT TCA CGA ATT TGC GT-3').

Mtt Assay

The cells transfected for 48 h were cultured in 96-well plates. The cells containing miR-1271-5p mimic or inhibitor were incubated at 24, 48, 72 h and 96 h. Then these cells were added with 20 μL MTT solutions (Thermo Fisher Scientific, Inc.) and incubated for 4 h at 37˚C. Next, we terminated the incubation and discarded culture supernatant. Finally, the absorbance at 490 nm (OD = 490 nm) was detected with a spectrophotometer.

Transwell Assays

Transwell chambers (8-μm pore size membranes) were employed to perform cell migration and invasion assays. The lower chamber was added with 10% FBS and incubated at 37 °C with 5% CO_2. Then the upper surface with matrigel (BD Biosciences, USA) was used for cell invasion which cell migration assay was conducted without matrigel. The transfected SKOV3 and OVCAR cells were cultured in the upper chamber with serum-free medium. 24 h later, the migrated or invasive cells were fixed with methanol and stained with crystal violet. Finally, we counted the number of removed cells using a microscope.

Dual Luciferase Assay

The wild or mutant type of 3’-UTR of E2F5 was inserted into the pmirGLO luciferase vector (Promega, Madison, USA) to perform luciferase reporter experiments. Then, wild or mutant type of 3’-UTR of E2F5 and miR-1271-5p mimic were transfected into SKOV3 and OVCAR cells. Subsequently, the Dual Luciferase Assay System (Promega, USA) was applied to analyze luciferase activity.

Western Blot Analysis

The protein samples were obtained using RIPA lysis buffer. Then the proteins were separated through a 10% SDS-PAGE and incubated with 5% non-fat milk in PVDF membranes at room temperature. Next we incubated the membranes overnight at 4℃ with E-cadherin, N-cadherin, vimentin and mTOR, GAPDH primary antibodies (1:1000; Abcam, USA). After washing, they were incubated with goat polyclonal anti-rabbit IgG secondary antibody (1:2000; Abcam, USA). Then, protein expression levels were measured by ECL (ECL, Pierce).

Statistical analysis

The data were analyzed by SPSS 19.0 and Graphpad Prism 6. The data was shown as mean ± SD analyzed by Student’s t test. The differences between the groups were calculated through $\chi 2$ test or Tukey’s one-way ANOVA. Kaplan-Meier analysis was applied to draw the survival curves, and log-rank test was used to compare the survival differences. Significant difference was defined at $P < 0.05$.

Results

MiR-1271-5p was downregulated in OC tissues

Primarily, the expression of miR-1271-5p was measured in OC tissues through qRT-PCR assay. The qRT-PCR assay suggested that miR-1271-5p expression was apparently reduced in OC tissues in comparison with that of normal tissues (Fig. 1A). In Table 1, we identified that low expression of miR-1271-5p was associated with lymph node metastasis ($P = 0.007$) and FIGO stage ($P = 0.035$). Therefore, we considered that miR-1271-5p was downregulated in OC which might involve in the tumorigenesis of OC. In addition, the results of Kaplan-Meier survival curve indicated that low expression of miR-1271-5p was correlated with poor prognosis of OC patients ($P = 0.0051$, Fig. 1B). According to above result, aberrant expression of miR-1271-5p might relate to the prognosis of OC patient.

Table 1
Relationship between miR-1271-5p expression and their clinic-pathological characteristics of OC patients.

Characteristics	Cases	miR-1271-5p		P-value
		High	Low	
Age (years)				0.073
≥ 60	25	11	14	
60	20	9	11	
Histological grading				0.118
1–2	28	13	15	
3	17	7	10	
Tumor size				0.18
< 5 cm	30	14	16	
≥ 5 cm	15	6	9	
FIGO stage				0.035*
I-II	33	12	21	
III-IV	12	8	4	
Lymph node metastasis				0.007*
No	35	14	21	
Yes	10	6	4	
Statistical analyses were performed by the χ2 test.				
*P0.05 was considered significant.				

MiR-1271-5p inhibited cell proliferation, migration and invasion in OC

Then, the miR-1271-5p expression level was examined in A2780, SKOV3, OVCAR and IOSE80 cell lines to further investigate its effect in OC. Consistent with the results of OC tissues, miR-1271-5p expression was apparently declined in A2780, SKOV3 and OVCAR cells contrast to IOSE80 cells (Fig. 2A). Then miR-1271-5p mimics or inhibitor was transfected into SKOV3 and OVCAR cells. We found that miR-1271-5p expression was distantly enhanced by miR-1271-5p mimics and was reduced by miR-1271-5p inhibitor

detected by qRT-PCR (Fig. 2B). Furthermore, overexpression of miR-1271-5p was found to repress the proliferation of SKOV3 and OVCAR cells whereas cell proliferation was promoted by downregulation of miR-1271-5p in SKOV3 and OVCAR cells (Fig. 2C, 2D). Besides that, the results of Transwell assay displayed that cell migration was inhibited by miR-1271-5p mimics when miR-1271-5p inhibitor significantly promoted cell migration in SKOV3 and OVCAR cells (Fig. 2E). Similarly, the same result of miR-1271-5p was also identified for cell invasion in SKOV3 and OVCAR cells with miR-1271-5p mimics or inhibitor (Fig. 2F). Taken together, overexpression of miR-1271-5p was verified to suppress cell proliferation, migration and invasion in OC.

E2F5 was a direct target of miR-1271-5p in OC cells

Subsequently, E2F5 was predicted as a target gene of miR-1271-5p by the database of TargetScan (http://www.targetscan.org/) (Fig. 3A). Then, we conducted luciferase reporter assay to confirm that prediction. As we predicted, the luciferase activity was reduced in SKOV3 and OVCAR cells with miR-1271-5p mimics and E2F5-Wt vector. But miR-1271-5p mimics were not found to change the luciferase activity of E2F5-Mut (Fig. 3B). Furthermore, we also found that E2F5 expression had a negative association with miR-1271-5p expression in OC tissues (P□0.0001, R^2 = 0.5541; Fig. 3C). Besides that, we observed the expression levels of E2F5 in SKOV3 and OVCAR cells with miR-1271-5p mimics or inhibitor. The results of qRT-PCR experiment showed that E2F5 was downregulated in SKOV3 and OVCAR cells with miR-1271-5p mimics (Fig. 3D) and upregulated in SKOV3 and OVCAR cells with miR-1271-5p inhibitor (Fig. 3E). Hence, E2F5 was verified as a direct target of miR-1271-5p and had negative correlation with miR-1271-5p expression.

Upregulation of E2F5 was identified in OC tissues

Next, alternation of E2F5 expression was identified in OC tissues. The qRT-PCR experiment showed that E2F5 expression was increased in the OC tissue (Fig. 4A). And upregulation of E2F5 was also observed in A2780, SKOV3, OVCAR cell lines in contrast to IOSE80 cells (Fig. 4B). Besides that, high E2F5 expression was found to predict poor prognosis of OC patients (P = 0.0092, Fig. 4C). Therefore, E2F5 was considered to influence the development of OC.

MiR-1271-5p negatively regulated EMT and mTOR pathway in OC

In addition, we investigated the effect of miR-1271-5p on EMT and mTOR pathway in OC. The western blot showed that miR-1271-5p overexpression promoted E-cadherin expression and suppressed N-cadherin and Vimentin expressions (Fig. 5A). Inversely, downregulation of miR-1271-5p blocked E-cadherin expression and enhanced N-cadherin and Vimentin expression level (Fig. 5B). Thus, we considered that overexpression of miR-1271-5p suppressed cell metastasis by regulating EMT. Besides

that, the protein expression of mTOR was examined in SKOV3 cells with miR-1271-5p mimics or inhibitor. The results indicated that upregulation of miR-1271-5p reduced the expression levels of p-mTOR, but no change was found for mTOR expression level (Fig. 5A). On the contrary, downregulation of miR-1271-5p enhanced p-mTOR expression level (Fig. 5B). In brief, miR-1271-5p was found to negatively regulate EMT and mTOR pathway in OC.

Discussion

The alternation of miRNAs expression has been demonstrated to participate in pathogenesis of human cancers including OC [24], [25]. In this study, miR-1271-5p expression was found to be decreased in OC tissues and cell lines. Downregulation of miR-1271-5p was identified to be associated with the poor prognosis of OC. And miR-1271-5p inhibited the proliferation, migration and invasion of OC cells through regulating E2F5 and negatively regulated the mTOR signaling pathway in OC. Therefore, miR-1271-5p was verified as a tumor suppressor in OC.

Recently, miR-1271 acting as a suppressive miRNA has been identified in several human cancers. Qin et al proposed that miR-1271 inhibited cellular proliferation in hepatocellular carcinoma [26] which was similar to our results in OC. And miR-1271 was also identified to suppress migration, invasion and EMT by targeting ZEB1 and TWIST1 in pancreatic cancer cells [27]. Here, the same effect of miR-1271-5p on the migration, invasion and EMT was found in OC as well. More importantly, Liu et al found that miR-1271 inhibited the growth OC by targeting CCNG1 [28] which was consistent with our findings. Besides that, miR-1271 was also reported to negatively regulate mTOR signaling in pancreatic cancer [29] as well as in OC in current research. In addition, low expression of miR-1271 had been reported to predict poor prognosis of patients with neuroglioma [30] as well as our findings. All these studies supported our conclusion about the role of miR-1271-5p in OC again. Furthermore, E2F5 was identified as a direct target of miR-1271-5p in OC.

E2F5 was reported to affect DNA synthesis, initiation of replication and cell-cycle [31]. Zhao et al. found that E2F5 functioned as an oncogene with copy number gain in prostate cancer [32] and we also found the carcinogenesis of E2F5 in OC in this study. Moreover, E2F5 was reported to be upregulated in human cancers, such as gastric cancer [33] and colorectal cancer [34]. And the upregulation of E2F5 was also identified in OC. Additionally, we also examined that high E2F5 expression was related to poor prognosis of OC. Same as our results, it was reported that upregulation of E2F5 resulted in a worse clinical outcome and poor prognosis of breast cancer [35]. Besides that, E2F5 had been verified as a direct target of several miRNAs and had negative association with their expression, such as miR-129-3p [36], miR-132 [37] and miR-613 [38]. In present research, miR-1271-5p was found to directly target E2F5 and was inversely regulated its expression in OC. These results showed that miR-1271-5p suppressed the development of OC, at least in part, through inhibiting E2F5 expression.

Conclusion

In this study, miR-1271-5p was downregulated in OC tissues which predicted poor prognosis of OC patient. Moreover, miR-1271-5p inhibited the development of OC through regulating E2F5 and negatively regulated the mTOR signaling pathway in OC. These findings might be benefit for the diagnosis and therapy of OC.

Declarations

Acknowledgements

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Funding

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

QL wrote the manuscript and analyzed the data. JS sorted out experimental data and performed the data analyses. XX contributed to the conception of the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The Third Affiliated Hospital of Soochow University (The First People's Hospital of Changzhou). Signed written informed consents were obtained from the patients and/or guardians.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA: a cancer journal for clinicians 2016;66:7-30.

[2] Jayson GC, Kohn EC, Kitchener HC, Ledermann JA. Ovarian cancer. Lancet 2014;384:1376-88.

[3] Permuth-Wey J, Sellers TA. Epidemiology of ovarian cancer. Methods in molecular biology 2009;472:413-37.

[4] Bristow RE. Surgical standards in the management of ovarian cancer. Current opinion in oncology 2000;12:474-80.

[5] Harries M, Gore M. Part II: chemotherapy for epithelial ovarian cancer-treatment of recurrent disease. The Lancet Oncology 2002;3:537-45.

[6] Waldron L, Haibe-Kains B, Culhane AC, Riester M, Ding J, Wang XV, et al. Comparative meta-analysis of prognostic gene signatures for late-stage ovarian cancer. Journal of the National Cancer Institute 2014;106.

[7] Liu J, Matulonis UA. New strategies in ovarian cancer: translating the molecular complexity of ovarian cancer into treatment advances. Clinical cancer research : an official journal of the American Association for Cancer Research 2014;20:5150-6.

[8] Calin GA, Croce CM. MicroRNA signatures in human cancers. Nature reviews Cancer 2006;6:857-66.

[9] Wang Y, Xu C, Wang Y, Zhang X. MicroRNA-365 inhibits ovarian cancer progression by targeting Wnt5a. American journal of cancer research 2017;7:1096-106.

[10] Qu W, Chen X, Wang J, Lv J, Yan D. MicroRNA-1 inhibits ovarian cancer cell proliferation and migration through c-Met pathway. Clinica chimica acta; international journal of clinical chemistry 2017;473:237-44.

[11] Wei J, Zhang L, Li J, Zhu S, Tai M, Mason CW, et al. MicroRNA-205 promotes cell invasion by repressing TCF21 in human ovarian cancer. Journal of ovarian research 2017;10:33.

[12] Liu H, Pan Y, Han X, Liu J, Li R. MicroRNA-216a promotes the metastasis and epithelial-mesenchymal transition of ovarian cancer by suppressing the PTEN/AKT pathway. OncoTargets and therapy 2017;10:2701-9.

[13] Liang HF, Zhang XZ, Liu BG, Jia GT, Li WL. Circular RNA circ-ABCB10 promotes breast cancer proliferation and progression through sponging miR-1271. American journal of cancer research 2017;7:1566-76.

[14] Li C, Jiang Y, Miao R, Qu K, Zhang J, Liu C. MicroRNA-1271 functions as a metastasis and epithelial-mesenchymal transition inhibitor in human HCC by targeting the PTP4A1/c-Src axis. International journal of oncology 2018;52:536-46.

[15] Li J, Xu J, Yan X, Jin K, Li W, Zhang R. Suppression of Capn4 by microRNA-1271 impedes the proliferation and invasion of colorectal cancer cells. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 2018;99:162-8.

[16] Lu G, Du L, Guo Y, Xing B, Lu J, Wei Y. Expression and role of microRNA-1271 in the pathogenesis of osteosarcoma. Experimental and therapeutic medicine 2018;15:1934-40.

[17] Wang Y, Xu L, Jiang L. miR-1271 promotes non-small-cell lung cancer cell proliferation and invasion via targeting HOXA5. Biochemical and biophysical research communications 2015;458:714-9.

[18] Sun J, Li H, Huo Q, Cui M, Ge C, Zhao F, et al. The transcription factor FOXN3 inhibits cell proliferation by downregulating E2F5 expression in hepatocellular carcinoma cells. Oncotarget 2016;7:43534-45.

[19] Li L, Wu C, Zhao Y. miRNA-34a enhances the sensitivity of gastric cancer cells to treatment with paclitaxel by targeting E2F5. Oncology letters 2017;13:4837-42.

[20] Xu H, Fei D, Zong S, Fan Z. MicroRNA-154 inhibits growth and invasion of breast cancer cells through targeting E2F5. American journal of translational research 2016;8:2620-30.

[21] Xu X, Cai N, Zhi T, Bao Z, Wang D, Liu Y, et al. MicroRNA-1179 inhibits glioblastoma cell proliferation and cell cycle progression via directly targeting E2F transcription factor 5. American journal of cancer research 2017;7:1680-92.

[22] Kropp J, Degerny C, Morozova N, Pontis J, Harel-Bellan A, Polesskaya A. miR-98 delays skeletal muscle differentiation by down-regulating E2F5. The Biochemical journal 2015;466:85-93.

[23] Ishimoto T, Shiozaki A, Ichikawa D, Fujiwara H, Konishi H, Komatsu S, et al. E2F5 as an independent prognostic factor in esophageal squamous cell carcinoma. Anticancer research 2013;33:5415-20.

[24] Lin Y, Xu T, Zhou S, Cui M. MicroRNA-363 inhibits ovarian cancer progression by inhibiting NOB1. Oncotarget 2017;8:101649-58.

[25] Shi H, Shen H, Xu J, Zhao S, Yao S, Jiang N. MiR-143-3p suppresses the progression of ovarian cancer. American journal of translational research 2018;10:866-74.

[26] Qin A, Zhu J, Liu X, Zeng D, Gu M, Lv C. MicroRNA-1271 inhibits cellular proliferation of hepatocellular carcinoma. Oncology letters 2017;14:6783-8.

[27] Liu H, Wang H, Liu X, Yu T. miR-1271 inhibits migration, invasion and epithelial-mesenchymal transition by targeting ZEB1 and TWIST1 in pancreatic cancer cells. Biochemical and biophysical research communications 2016;472:346-52.

[28] Liu X, Ma L, Rao Q, Mao Y, Xin Y, Xu H, et al. MiR-1271 Inhibits Ovarian Cancer Growth by Targeting Cyclin G1. Medical science monitor : international medical journal of experimental and clinical research 2015;21:3152-8.

[29] Xie F, Huang Q, Liu CH, Lin XS, Liu Z, Liu LL, et al. MiR-1271 negatively regulates AKT/MTOR signaling and promotes apoptosis via targeting PDK1 in pancreatic cancer. European review for medical

and pharmacological sciences 2018;22:678-86.

[30] Xiang XJ, Deng J, Liu YW, Wan LY, Feng M, Chen J, et al. MiR-1271 Inhibits Cell Proliferation, Invasion and EMT in Gastric Cancer by Targeting FOXQ1. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 2015;36:1382-94.

[31] Trimarchi JM, Lees JA. Sibling rivalry in the E2F family. Nature reviews Molecular cell biology 2002;3:11-20.

[32] Zhao J, Wu XY, Ling XH, Lin ZY, Fu X, Deng YH, et al. Analysis of genetic aberrations on chromosomal region 8q21-24 identifies E2F5 as an oncogene with copy number gain in prostate cancer. Medical oncology 2013;30:465.

[33] Yao YL, Wu XY, Wu JH, Gu T, Chen L, Gu JH, et al. Effects of microRNA-106 on proliferation of gastric cancer cell through regulating p21 and E2F5. Asian Pacific journal of cancer prevention : APJCP 2013;14:2839-43.

[34] Lu G, Sun Y, An S, Xin S, Ren X, Zhang D, et al. MicroRNA-34a targets FMNL2 and E2F5 and suppresses the progression of colorectal cancer. Experimental and molecular pathology 2015;99:173-9.

[35] Umemura S, Shirane M, Takekoshi S, Kusakabe T, Itoh J, Egashira N, et al. Overexpression of E2F-5 correlates with a pathological basal phenotype and a worse clinical outcome. British journal of cancer 2009;100:764-71.

[36] Fang DZ, Wang YP, Liu J, Hui XB, Wang XD, Chen X, et al. MicroRNA-129-3p suppresses tumor growth by targeting E2F5 in glioblastoma. European review for medical and pharmacological sciences 2018;22:1044-50.

[37] Tian H, Hou L, Xiong YM, Huang JX, Zhang WH, Pan YY, et al. miR-132 targeting E2F5 suppresses cell proliferation, invasion, migration in ovarian cancer cells. American journal of translational research 2016;8:1492-501.

[38] Zhang Y, Zhu X, Zhu X, Wu Y, Liu Y, Yao B, et al. MiR-613 suppresses retinoblastoma cell proliferation, invasion, and tumor formation by targeting E2F5. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 2017;39:1010428317691674.

Figures

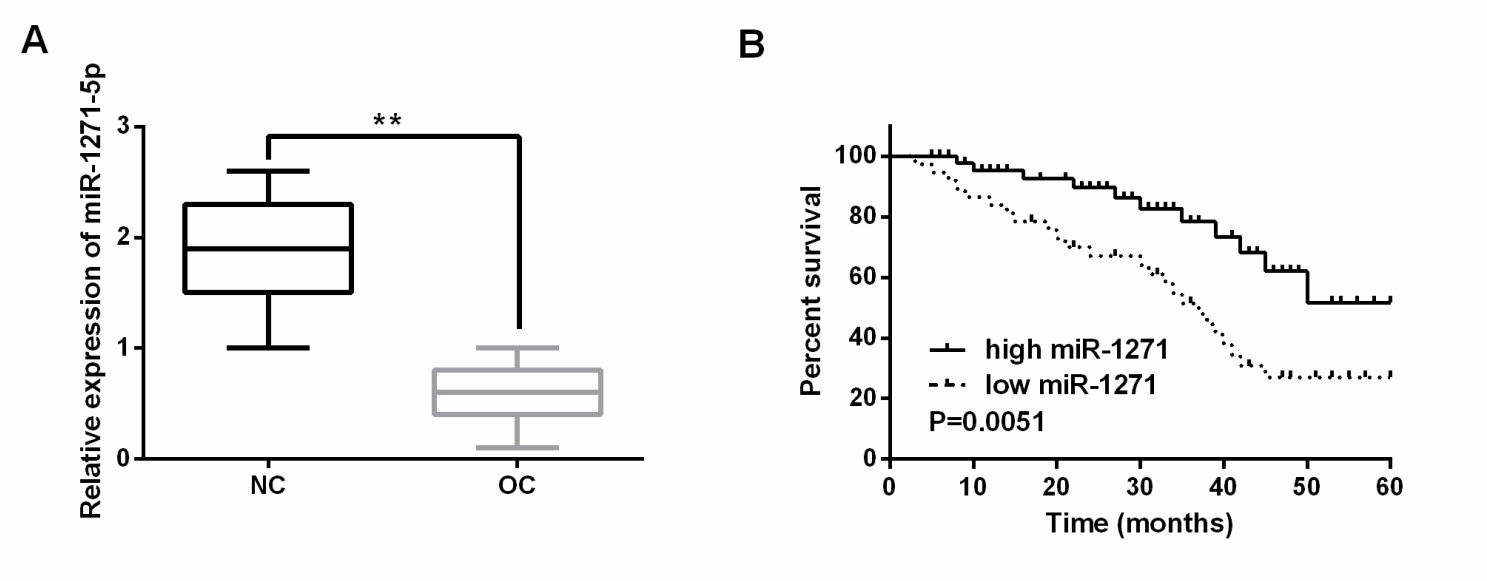

Figure 2

MiR-1271-5p was downregulated in OC tissues. (A) The expressions of miR-1271-5p in OC tissues detected via qRT-PCR. (B) Lower miR-1271-5p expression was related to shorter overall survival in OC patients. **P < 0.01.

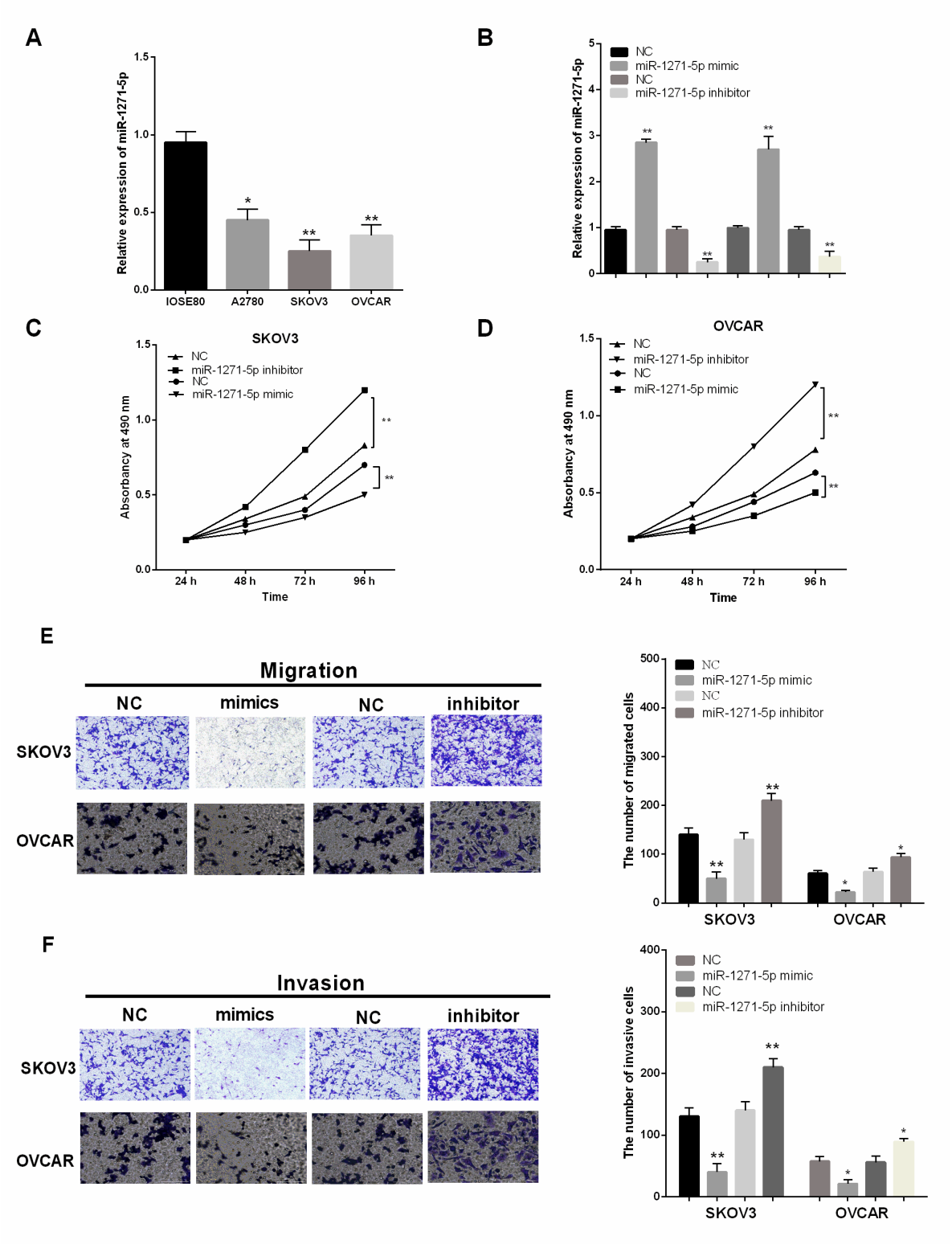

Figure 4

MiR-1271-5p inhibited cell proliferation, migration and invasion in OC. (A) The miR-1271-5p expression in A2780, SKOV3, OVCAR and IOSE80 cell lines. (B) The miR-1271-5p expression was examined in SKOV3 and OVCAR cells with miR-1271-5p mimics or inhibitor via qRT-PCR. (C, D) The cell proliferation was measured in cells containing miR-1271-5p mimics or inhibitor via MTT assay. (E, F) Cell migration and

invasion analysis in cells containing miR-1271-5p mimics or inhibitor was detected by Transwell assay. * P <0.05, ** P <0.01.

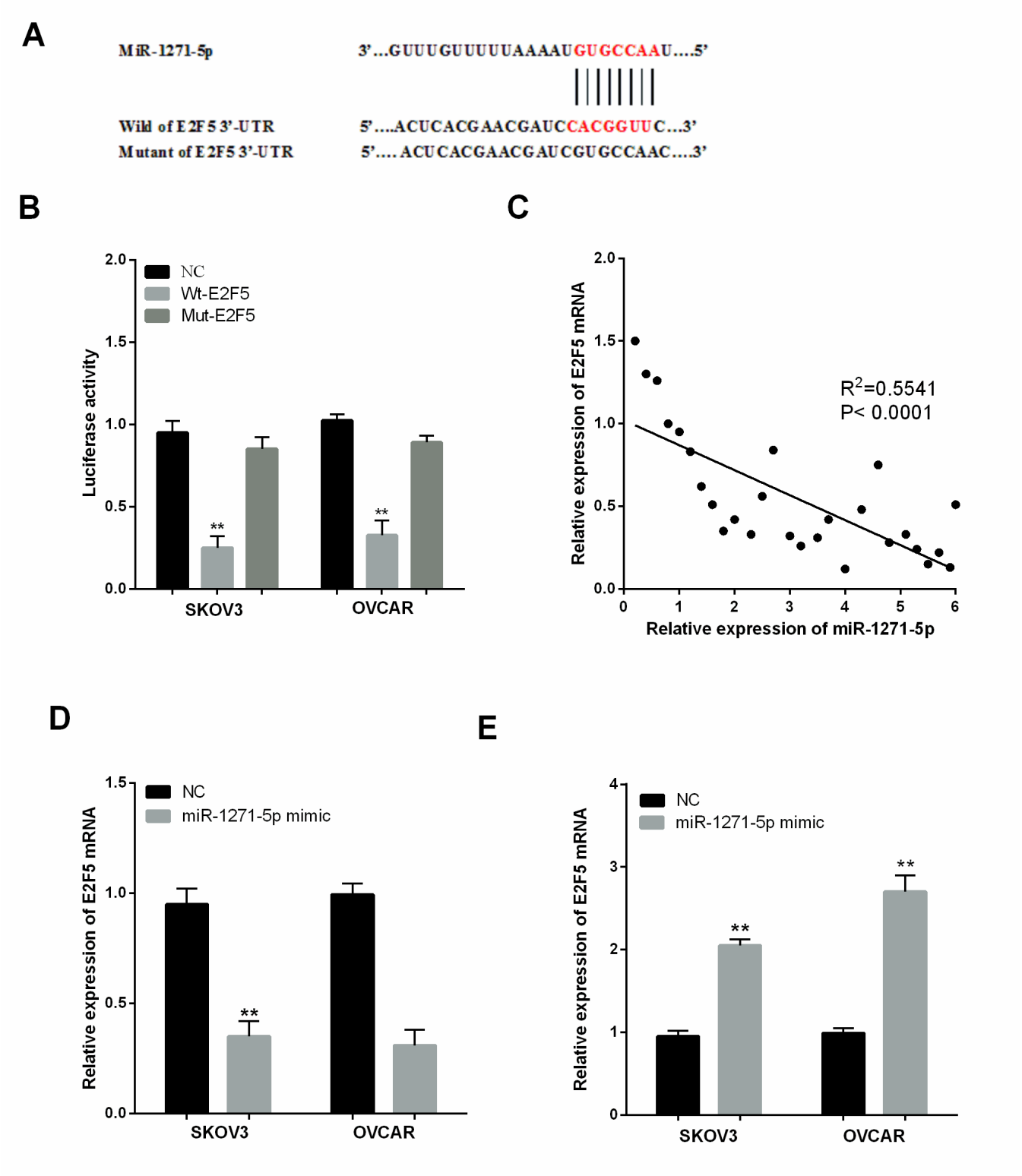

Figure 5

E2F5 was a direct target of miR-1271-5p in OC cells. (A) The binding sites of miR-1271-5p on the 3'-UTR of E2F5 (B) Luciferase reporter assay (C) The correlation between miR-1271-5p and E2F5. (D, E) The

expression of E2F5 were observed in SKOV3 and OVCAR cells containing miR-1271-5p mimics or inhibitor ** P <0.01.

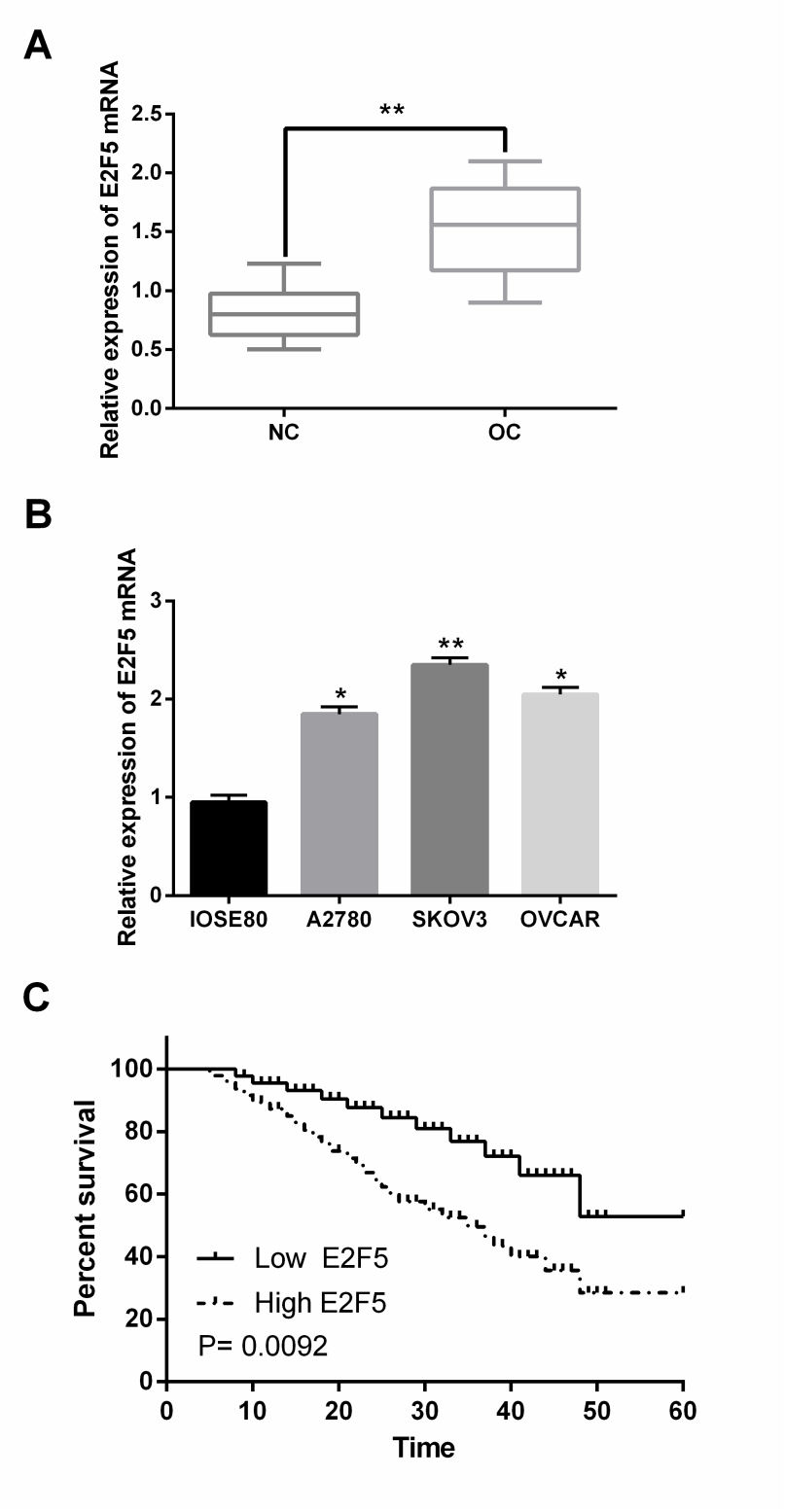

Figure 8

Upregulation of E2F5 was identified in OC tissues. (A) The expressions of E2F5 in OC tissues detected via qRT-PCR. (B) The E2F5 expression in A2780, SKOV3, OVCAR and IOSE80 cell lines. (C) High E2F5 expression was related to shorter overall survival in OC patients. *P < 0.05, **P < 0.01.

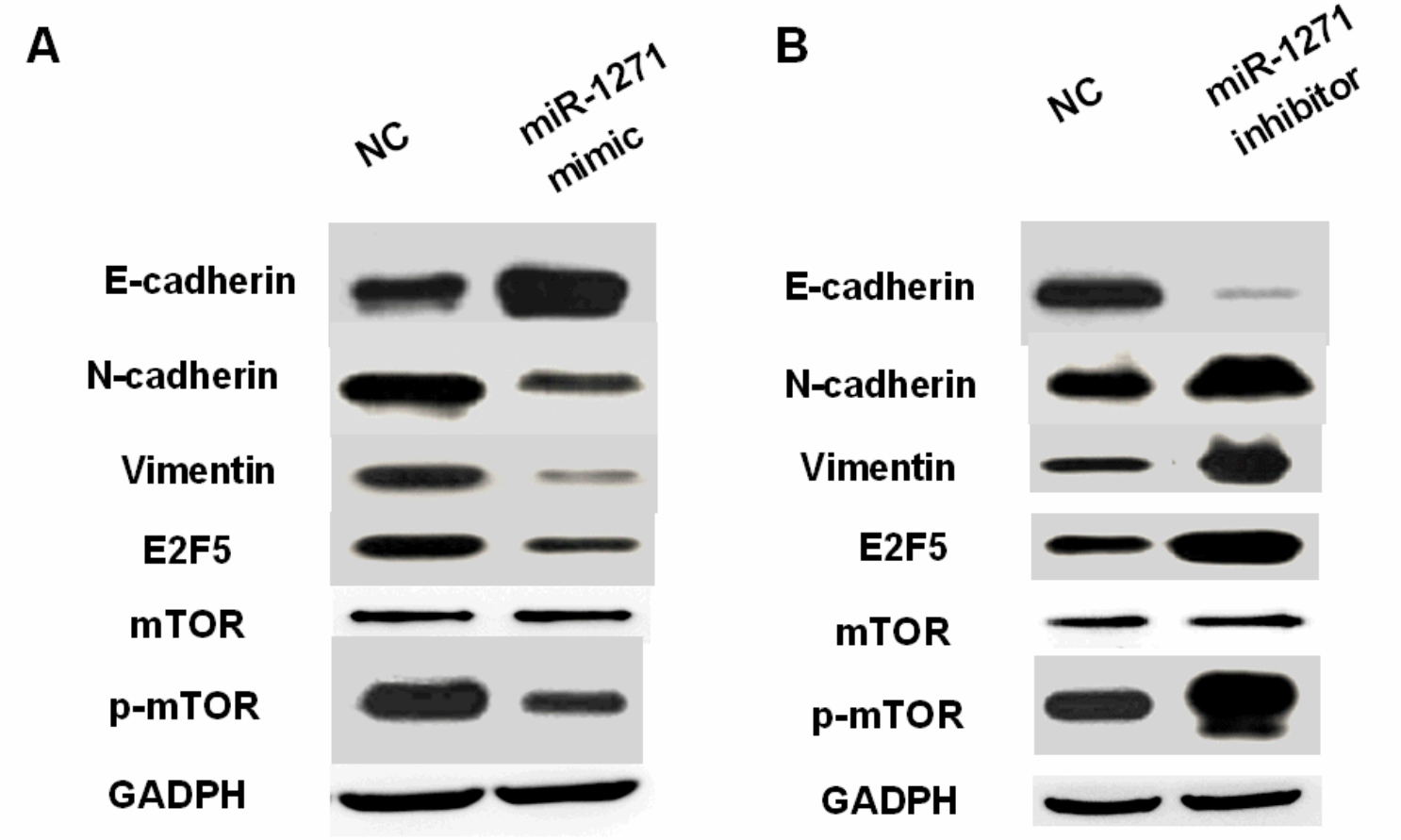

Figure 10

MiR-1271-5p negatively regulated EMT and mTOR pathway in OC. (A, B) Western blot analysis of E-cadherin, N-cadherin, Vimentin, mTOR and p-mTOR in SKOV3 cells contained miR-1271-5p mimics or inhibitor.