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Long non-coding RNA OR3A4 promotes metastasis of ovarian cancer *via* inhibiting KLF6

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Abstract. – OBJECTIVE: Recently, long non-coding RNAs (lncRNAs) have attracted much attention for their roles in tumor progression. The aim of this study was to investigate the exact role of lncRNA OR3A4 in the development of ovarian cancer (OC), and to explore the possible underlying mechanism.

PATIENTS AND METHODS: OR3A4 expression in OC tissue samples was detected by Real-time quantitative polymerase chain reaction (qRT-PCR). Moreover, wound healing a[illegible] and transwell assay were performed to e[illegible] the effect of OR3A4 on the metastasis [illegible] OC. Furthermore, the underlying mechanis[illegible]as explored by RT-qPCR and Western blot as[illegible]

RESULTS: The expression level of OR3A[illegible] OC samples was significantly hi[illegible]r than t[illegible] of adjacent tissues. Moreo[illegible]migratio[illegible] and invasion were signifi[illegible]ly re[illegible]sed after OR3A4 knockdown *i[illegible]tro*. Mo[illegible]ver, the mRNA and protein exp[illegible]s of [illegible] like factor 6 (KLF6) were [illegible] after knockdown of [illegible]A4. F[illegible]ore, the expression level of [illegible]6 was neg[illegible] correlated with the exp[illegible] of OR3A4 i[illegible]issues.

CONCLUSIO[illegible] [illegible] results sh[illegible]ed that OR3A4 could [illegible]hance [illegible]ll metastasis and invasion *via* [illegible]ppressing [illegible] Moreover, OR3A4 might b[illegible] potential therap[illegible] target for OC.

Key [illegible]ds
[illegible]onc[illegible]g RNA, OR3A4, Ovarian cancer (OC), [illegible]

Introduction

[illegible]varian cancer (OC) is one of the most com[illegible] [illegible]alignancies in women worldwide. It is repo[illegible]ed that approximately 22,280 patients are newly diagnosed of OC in US. Meanwhile, almost 15,5[illegible] ar[illegible]mated to die of OC in the same year[illegible]. Due to [illegible]ailable tests, OC is often diagnosed at advanced [illegible] making it one of the le[illegible]uses of cancer-[illegible]ed death in females. T[illegible] main interventions for OC include surgery, [illegible]emotherapy a[illegible] radiotherapy. However, most [illegible]ents develop [illegible]stance to chemotherapy or rela[illegible] after surge[illegible] Furthermore, the prognosis of pati[illegible] [illegible] is still dismal, with 5-year survival rat[illegible]y 30%[1,2]. Thus, the severe situation [illegible]ighlights the urgency of early detection and new [illegible]tic treatment for OC patients. Researches [illegible]ve [illegible]icated that long non-coding RNAs (lncRNAs) are closely involved in a variety of cellular activities. For example, lncRNA MSTO2P facilitates the proliferation and colony formation of gastric cancer cells indirectly by regulating the expression of miR-335[3]. LncRNA SNHG7 promotes epithelial-to-mesenchymal transition and tumor proliferation in osteosarcoma by regulation of miR-34a Signals[4]. LncRNA PCAT-1 plays an important role in the tumorigenesis of hepatocellular carcinoma *via* modulating TP53-miR-215-PCAT-1-CRKL axis[5]. Zhang et al[6] has indicated that up-regulation of lncRNA FENDRR inhibits the proliferation and malignancy of non-small cell lung cancer by serving as a sponge of miR-761. In addition, lncRNA RUNX1-IT1 inhibits the migration and proliferation of colorectal cancer, acting as a tumor suppressor[7]. However, the exact role of lncRNA OR3A4 in the progression of OC, as well as the underlying molecular mechanism has not been fully elucidated. In this study, we found that OR3A4 was highly expressed in OC tissues. Moreover, OR3A4 significantly promoted the migration and invasion of OC cells *in vitro*. Moreover, our further experiments explored the underlying mechanism of how OR3A4 functioned in OC development.

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Patients and Methods

Cell Lines and Clinical Samples

A total of 52 OC tissues were collected from patients who received surgery at Xinhua Hospital Affiliated to Dalian University between June 2015 and July 2018. No radiotherapy or chemotherapy was performed for any patients before the operation. All fresh tissues were stored at -80°C for subsequent use. This study was approved by the Ethics Committee of Xinhua Hospital Affiliated to Dalian University. Signed written informed consents were obtained from all participants before the study and operation.

Cell Culture

Three human OC cell lines (A2780, SKOV3 and OVCAR-3) and one normal ovarian cell line (ISOE80) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Life Technologies, Gaithersburg, MD, USA) and penicillin. Besides, all cells were maintained in an incubator with 5% CO_2 at 37°C.

Cell Transfection

After synthesis, cDNA oligonucleoti[illegible]targeting OR3A4 (OR3A4/shRNA) were clone[illegible]o pGPH1/Neo vector (GenePharma, Shanghai, [illegible]na)). 293T cells were used for pa[illegible]ing OR3A[illegible] shRNA and empty vector, wh[illegible]en transfected into OC cells. 48 h [illegible]r cell [illegible]fection, the expression level of O[illegible] in cells [illegible]s detected using Real-time quanti[illegible]oly[illegible] reaction (qRT-PCR).

RNA Extracti[illegible] and RT-qPC[illegible]

Total RNA was extr[illegible]according to the instructions [illegible]TRIzol reagen[illegible]itrogen, Carlsbad, CA, US[illegible] Subsequently, extra[illegible] total RNA was reve[illegible]ranscrib[illegible]d to complementary deoxyribo[illegible]leic [illegible]s (cDNAs) in strict accordance with [illegible]anscript[illegible] Kit (TaKaRa Biotech[illegible]ology C[illegible]d, D[illegible]n, China). Primers used [illegible] study [illegible] follows: OR3A4, forward 5′-C[illegible]ATCCC[illegible]CTCTAAGAA-3′ and reverse 5′-A[illegible]TCTGCAAAAACGTGCTG-3′; glyceral[illegible]sphate dehydrogenase (GAPDH), [illegible]ward 5′-CCAAAATCAGATGGGGCAATGCT-[illegible]′ and reverse 5′-TGATGGCATGGACTGTGG[illegible]TCA-3′. Thermal cycle was as follows: 30 s at 95[illegible]C, 5 s for 40 cycles at 95°C, and 35 s at 60°C.

Western Blot Analysis

Total proteins were extracted from cells *via* radioimmunoprecipitation assay (RI[illegible] fer. After that, the concentration [illegible] protein was quantified by the [illegible]inchoninic acid method (Beyotime, Shang[illegible] China). Target protein samples were separa[illegible] sodium dodecyl sulphate-polyacry[illegible]mide [illegible]lectrophoresis (SDS-PAGE) [illegible] transfer[illegible] polyvinylidene difluo[illegible] (PVDF) mem[illegible] (Millipore, Billeric[illegible] MA, U[illegible]). Next, [illegible]e membranes were in[illegible]d [illegible]h prim[illegible]y antibodies of rabb[illegible]nti-[illegible] (Cell [illegible]naling Technology, [illegible], Danvers, [illegible] US[illegible] and rabbit anti-KL[illegible]uppel-like fa[illegible]) (Cell Signaling Te[illegible]o[illegible]CST, Danve[illegible], MA, USA). On the next day, t[illegible]mbranes were incubated with corresponding s[illegible]ary antibodies. Chemi[illegible]scent film was [illegible]lied for assessment [illegible] protein expression with Image J software [illegible]IH, Bethesda[illegible]MD, USA).

W[illegible]nd Heali[illegible] Assay

C[illegible] were [illegible] seeded into 6-well plates and cultured [illegible]DMEM medium overnight. After [illegible]ratching with a plastic tip, the cells were cul[illegible]erum-free DMEM medium. Wound clo[illegible] viewed 48 h later. Each assay was independently repeated in triplicate.

Transwell Assay

5×10^4 cells in 200 μL serum-free DMEM were seeded into the upper chamber of an 8-μm pore size insert (Millipore, Billerica, MA, USA) coated with or without 50 μg Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). Meanwhile, the lower chamber was added with DMEM and FBS. 48 h later, after wiped by cotton swab, the top surface of the chamber was immersed with precooling methanol for 10 min. Then the cells were stained with crystal violet for 30 min. Three fields randomly selected for each sample, and the number of migrated and invaded cells was counted.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (IBM, Armonk, NY, USA) was used for all statistical analysis. Data were presented as mean ± SD (Standard Deviation). Student's *t*-test was utilized to compare the difference between two groups. $p<0.05$ was considered statistically significant.

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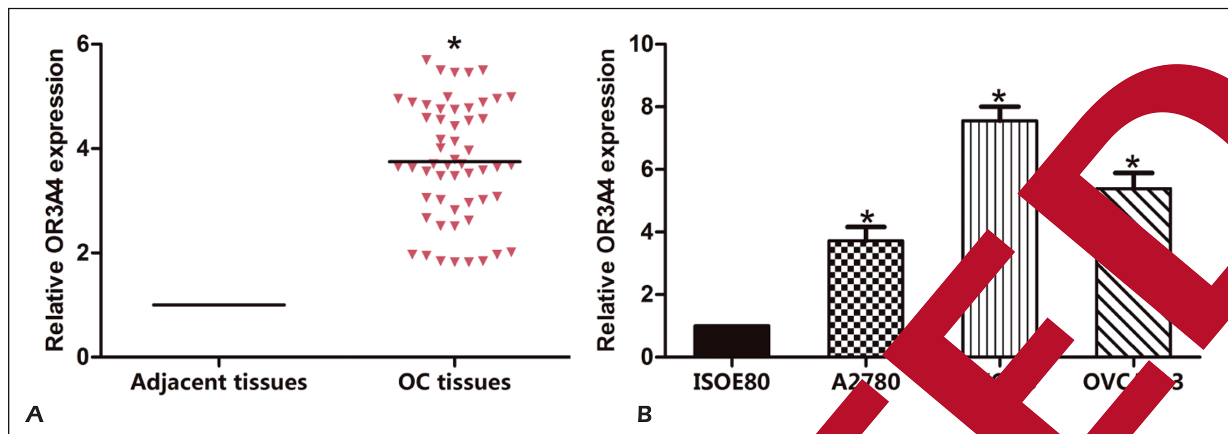


Figure 1. Expression level of OR3A4 was significantly increased in OC tissues a[illegible]nes. **A**, OR3A4 [illegible]n was significantly increased in OC tissues when compared with adjacent tissues. **B**, Expressi[illegible]ev[illegible]R3A4 relative t[illegible]APDH were determined in human OC cell lines and ISOE80 by RT-qPCR. Data were present[illegible]as mean [illegible]ard error of the mean. *$p<0.05$.

Results

Expression of OR3A4 in OC Tissues and Cells

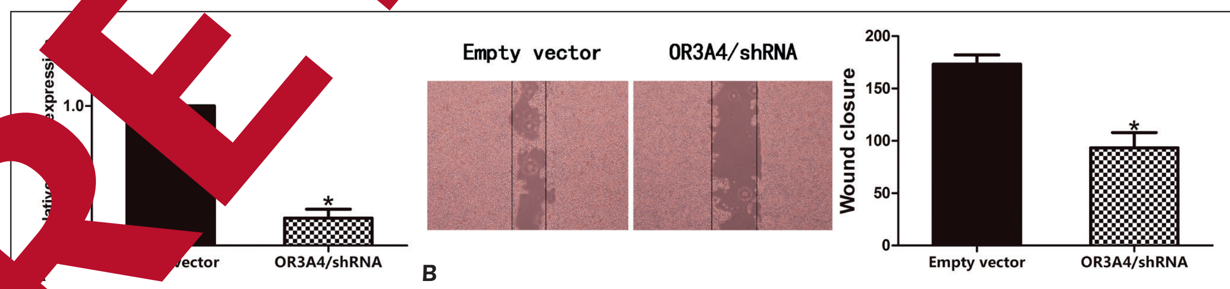
First, RT-qPCR was conducted to detect OR3A4 expression in 52 OC patients' tissues and 3 OC cell lines. Results demonstrate[illegible] OR3A4 expression was significantly up-r[illegible] in OC tissue samples (Figure 1A). Besides, [illegible]expression of OR3A4 in OC cells was signifi[illegible] higher than that of ISOE80 cells (Figure 1B).

Knockdown of OR3A4 In[illegible] Migration and Invasion [illegible] OC C[illegible]

In our study, SKOV3[illegible]ls were [illegible]osen for knockdown of OR3A4. Af[illegible]ansfe[illegible] CR was utilized to ve[illegible] the e[illegible]on of OR3A4 (Figure 2A). Resu[illegible]f wound h[illegible] assay revealed that after [illegible]4 knockdown, [illegible] migration ability of [illegible] cel[illegible] significantly repressed (Figure 2B). Meanwhile, [illegible]swell assay also indi[illegible]at after OR3A4 [illegible]wn-expressed in OC [illegible]s, the number of migrated cells was remarkably [illegible]reased (Figur[illegible]A and 3B). Furthermore, the in[illegible]on of OC cel[illegible]as remarkably decreased after O[illegible]4 knockdo[illegible] (Figure 3C and 3D).

Interact[illegible]etween KLF6 [illegible]nd OR3A4 in OC

[illegible]CR results showed that compared with [illegible]mpty [illegible]ctor group, the expression level of KLF6 in OC cells of OR3A4/shRNA group was significantly higher (Figure 4A). Western blot assay found that after OR3A4 knockdown, the protein expression of KLF6 was significantly upregulated (Figure 4B). Our results further illustrated that KLF6 expression in OC tissues was significantly lower when compared with that of adjacent tissues (Figure 4C). Subsequent correlation analysis demonstrated that the expression level of KLF6 was negatively correlated with OR3A4 expression in OC tissues (Figure 4D).



[illegible] **2.** Wound healing assay showed that knockdown of OR3A4 inhibited OC cell migration. **A**, OR3A4 expression in OC [illegible] transfected with OR3A4/shRNA and empty vector was detected by RT-qPCR, respectively. GAPDH was used as an interna[illegible] control. **B**, Wound-healing assay showed that knockdown of OR3A4 significantly repressed the migration of OC cells. The results represented the average of three independent experiments (mean ± standard error of the mean). *$p<0.05$.

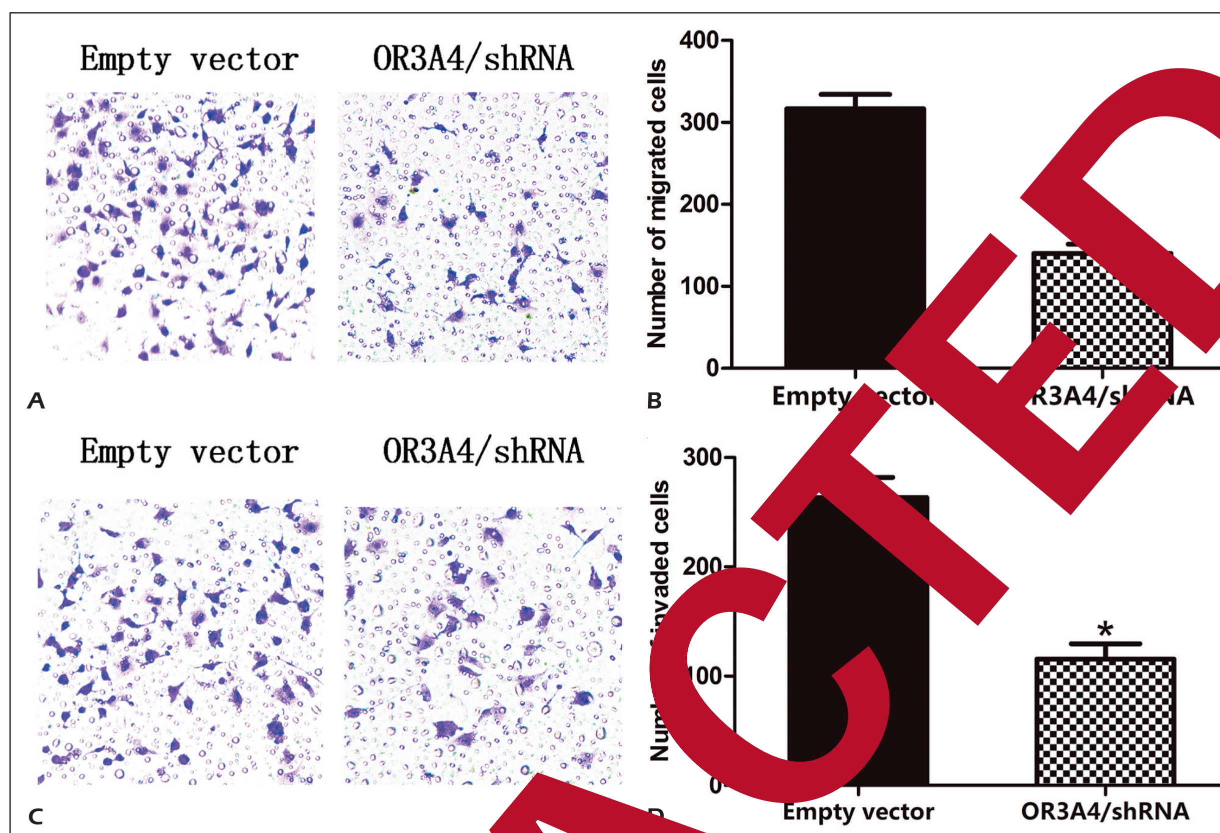


Figure 3. Transwell assay showed that knockdown of OR3A4 inhibited OC cell migration and invasion. **A**, Representative pictures of migrated cells in OR3A4/shRNA group and empty vector group. **B**, The number of migrated cells was significantly decreased after knockdown of OR3A4 in OC cells. **C**, Representative pictures of invaded cells in OR3A4/shRNA group and empty vector group. **D**, Transwell assay showed that number of invaded cells was significantly decreased after knockdown of OR3A4 in OC cells. The results represent the average of three independent experiments (mean ± standard error of the mean). *p<0.05.

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Discussion

Evidence indicates that lncRNA is an important regulator in the progression of OC, which can be used as a potential marker and therapeutic target for OC. For instance, silence of lncRNA MNX1-AS1 suppresses the proliferation and migration of OC cells, which may be a potential target for OC. LncRNA BACE1-AS inhibits the proliferation and invasion of OC stem cells, functioning as a novel target for OC treatment[9]. LncRNA HOXA1 facilitates the proliferation and migration of serous OC, which is associated with the prognosis of patients[10]. Through regulation of epithelial-mesenchymal transition, downregulation of lncRNA SP[illegible] enhances the metastasis of OC[11]. In addition, lncRNA ElncRNA1, as an oncogene in the proliferation of epithelial OC cells, is significantly upregulated by estrogen[12]. Olfactory receptor family 3 subfamily A member 4 (OR3A4) (Accession Number: NR_024128.1) is a novel lncRNA that has been widely explored recently. It is abnormally expressed in several cancers, which is related to cancer progression. For instance, upregulation of OR3A4 facilitates the proliferation and metastasis of breast cancers *via* epithelial-mesenchymal transition[13]. OR3A4 promotes the proliferation and tumorigenesis of gastric cancer, serving as a potential therapeutic strategy[14]. In this study, we found that OR3A4 was significantly upregulated in OC tissues and cells. Besides, after OR3A4 knockdown, the migration and invasion of OC cells were found remarkably inhibited. All above results indicated that OR3A4 promoted tumorigenesis of OC and might act as an oncogene. KLF6 (Krüppel-like factor 6) functions as a tumor suppressor in a diverse of tumors by regulating various biological processes. For example, through attenuating the activity of MMP-9 and the expression level of mesenchymal markers, overexpression of KLF6 inhibits the migration and invasion of oral cancer cells[15]. KLF6-E2F1 axis is activated in aggressive clear cell renal

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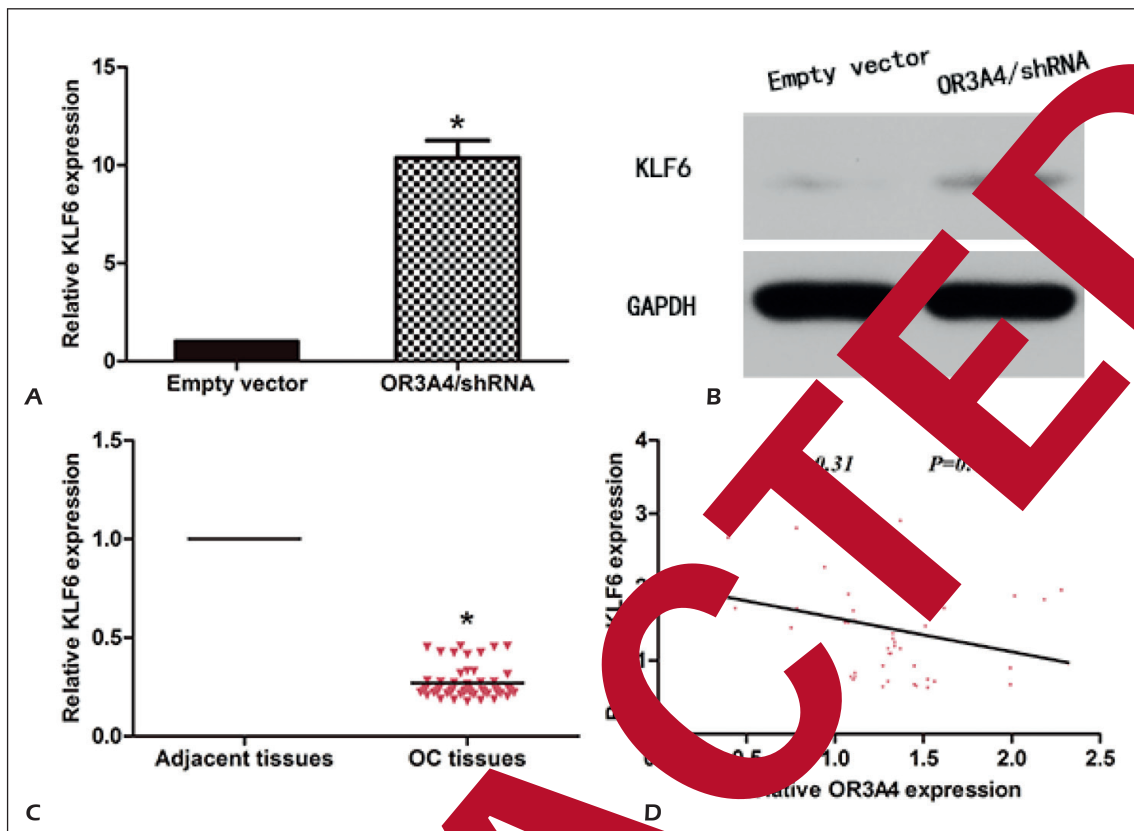

Figure 4. Interaction between OR3A4 and KLF6. A [illegible] qPCR re[illegible] that KLF6 expression in OR3A4/shRNA group was significantly higher than empty vector group. **B**, [illegible]ern b[illegible]ssay [illegible]ealed that protein expression of KLF6 in OR3A4/shRNA group was remarkably increased when compar[illegible] [illegible]pty vector group. **C**, KLF6 was significantly downregulated in OC tissues when compared with adja[illegible] tissues. **D**, [illegible] correlation between the expression level of KLF6 and OR3A4 in OC tissues. The results represente[illegible] of three [illegible]endent experiments. Data were presented as mean ± standard error of the mean. *p<0.05.

cell carcinoma. Meanwhi[illegible] [illegible]F6 f[illegible] tumor suppressor *via* [illegible]nscrip[illegible] [illegible]epression of E2F1[16]. KLF6 is d[illegible]ed in glio[illegible]as, which is related to po[illegible]nosis of pat[illegible] through targeting KL[illegible] M[illegible]er, by targeting KLF6, overexpression of miR-[illegible] [illegible]omotes growth, proliferation [illegible] migration of e[illegible]ial OC[18]. Furthermore, K[illegible]6 expression could [illegible] upregulated after knoc[illegible]wn of OR3A4. Moreover, KLF6 expressio[illegible] [illegible]C ti[illegible]s was negatively correlated with OR3A[illegible]ion. All [illegible] above results suggested that OR3A[illegible] [illegible]ght p[illegible]ote tumorigenesis of OC [illegible]geting [illegible]

Conclusions

[illegible]e showed that OR3A4 could enhance OC cell m[illegible]n and invasion through targeting KLF6. Thes[illegible] findings suggested that OR3A4 might contribute to therapy for OC as a candidate target.

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Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62: 10-29.
2) Brinkhuis M, Izquierdo MA, Baak JP, van Diest PJ, Kenemans P, Scheffer GL, Scheper RJ. Expression of multidrug resistance-associated markers, their relation to quantitative pathologic tumour characteristics and prognosis in advanced ovarian cancer. Anal Cell Pathol 2002; 24: 17-23.

3) Li H, Zhu H, Zhou Y, Wang H, Niu Z, Shen Y, Lv L. Long non-coding RNA MSTO2P promotes the proliferation and colony formation in gastric cancer by indirectly regulating miR-335 expression. Tumour Biol 2017; 39: 1393384158.
4) Deng Y, Zhao F, Zhang Z, Sun F, Wang M. Long noncoding RNA SNHG7 promotes the tumor growth and epithelial-to-mesenchymal transition via regulation of miR-34a signals in osteosarcoma. Cancer Biother Radiopharm 2018; 33: 365-372.
5) Ren Y, Shang J, Li J, Liu W, Zhang Z, Yuan J, Yang M. The long noncoding RNA PCAT-1 links the microRNA miR-215 to oncogene CRKL-mediated signaling in hepatocellular carcinoma. J Biol Chem 2017; 292: 17939-17949.
6) Zhang MY, Zhang ZL, Cui HX, Wang RK, Fu L. Long non-coding RNA FENDRR inhibits NSCLC cell growth and aggressiveness by sponging miR-761. Eur Rev Med Pharmacol Sci 2018; 22: 8324-8332.
7) Shi J, Zhong X, Song Y, Wu Z, Gao P, Zhao J, Sun J, Wang J, Liu J, Wang Z. Long non-coding RNA RUNX1-IT1 plays a tumour-suppressive role in colorectal cancer by inhibiting cell proliferation and migration. Cell Biochem Funct 2019; 37: 11-20.
8) Lv Y, Li H, Li F, Liu P, Zhao X. Long Noncoding RNA MNX1-AS1 Knockdown inhibits cell proliferation and migration in ovarian cancer. Cancer Biother Radiopharm 2017; 32: 91-99.
9) Chen Q, Liu X, Xu L, Wang Y, Wang S, Li [illegible] Y, Liu T. Long non-coding RNA BACE1[illegible] is a novel target for anisomycin-mediated su[illegible]s-sion of ovarian cancer stem cell proliferatio[illegible] invasion. Oncol Rep 2016; 35: 1916-1924.
10) Yim GW, Kim HJ, Kim LK, Kim S[illegible] S, Nam [illegible] Kim YT. Long non-coding RN[illegible] antisens[illegible] promotes cell proliferation [illegible] inva[illegible] and predicts patient prognosis i[illegible]erous ova[illegible]n cancer. Cancer Res Treat 201[illegible]656-66[illegible]
11) Yu J, Han Q, Cui Y. Decreased long non-coding RNA SPRY4-IT1 contributes to ovarian cancer cell metastasis partly via affecting [illegible]al-mesenchymal transition. Tumour Bi[illegible] 1393380535.
12) Qiu JJ, Zhang XD, Tang XY, Zheng [illegible] Zhang Y, Hua KQ. ElncRNA1, a long non-c[illegible] RNA that is transcriptionally induced by oe[illegible] promotes epithelial ovarian cancer cell pr[illegible]n. Int J Oncol 2017; 51: 507-514[illegible]
13) Liu G, Hu X, Zhou [illegible]ong non-cod[illegible] OR3A4 promotes p[illegible]eration and migra[illegible] breast cancer. Bi[illegible] Pharm[illegible]other 2017; [illegible]6: 426-433.
14) Guo X, Yang Z[illegible] Zhi Q[illegible] Guo L, [illegible] Miao R, Shi Y, Ku[illegible]Y. Long [illegible]ding R[illegible] OR3A4 promotes [illegible]astasis and t[illegible] in gastric cancer. [illegible]rget 2016; 7: 3[illegible]0294.
15) Hsu L[illegible] Lai HW, Hs[illegible]T, Sung WW, Hsieh [illegible], Wu C[illegible]M, Chen MK, Lo YS, Chen CJ. KLF6 inhibited ora[illegible]er migration and invasion [illegible]nregulation o[illegible]nchymal markers and [illegible]on of MMP-9 acti[illegible]es. Int J Med Sci 2017; 14: 530-535.
[illegible]) Gao Y, Li H, [illegible] X, Fan Y, Ni D, Zhang Y, Huang Q, Liu K, Li [illegible]ang L, Gu L, Yao Y, Ai Q, Du Q, [illegible]ong E, Zhang [illegible] KLF6 suppresses metastasis of [illegible]r cell re[illegible]cell carcinoma via transcriptional [illegible]E2F1. Cancer Res 2017; 77: 330-342.
17) Masilamani AP, Ferrarese R, Kling E, Thudi NK, Kim [illegible] Scholtens DM, Dai F, Hadler M, Unterkircher [illegible]tania L, Weyerbrock A, Prinz M, Gillespie GY, Harsh GI, Bredel M, Carro MS. KLF6 depletion promotes NF-kappaB signaling in glioblastoma. Oncogene 2017; 36: 3562-3575.
18) Zhang S, Zhang JY, Lu LJ, Wang CH, Wang LH. MiR-630 promotes epithelial ovarian cancer proliferation and invasion via targeting KLF6. Eur Rev Med Pharmacol Sci 2017; 21: 4542-4547.

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