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# Long non-coding RNA DANCR upregulates IGF2 expression and promotes ovarian cancer progression

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**Abstract. – OBJECTIVE:** Recent studies have revealed the important role of long non-coding RNA (lncRNAs) in the development of malignant tumors. In this work, we explored the exact role of lncRNA DANCR in ovarian cancer progression and the underlying mechanism.

**PATIENTS AND METHODS:** Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was used to detect DANCR expression in both ovarian cancer cells and tissue samples. Subsequently, cell proliferation assay and transwell assay were conducted. Furthermore, the underlying mechanism was explored via qRT-PC[illegible] Western blot assay.

**RESULTS:** The expression of DANCR in [illegible]rian cancer samples was significantly highe[illegible] that of the corresponding normal tissues. [illegible] DANCR overexpression *in vitr*[illegible] prolife[illegible]tion, invasion and migration [illegible] cancer cells were markedly promot[illegible]on, both the mRNA and protein ex[illegible]ion le[illegible] insulin-like growth factor 2 [illegible] upregulated after DA[illegible]CR [illegible]ssion[illegible]thermore, the resul[illegible]und th[illegible] expression level of IGF2 was [illegible]vely corre[illegible]ith DANCR expression [illegible] cancer t[illegible].

**CONCLUSI[illegible]** [illegible]udy, we revealed that DANCR could enhanc[illegible]roliferation, migration and [illegible]sion capac[illegible] ovarian cancer cells b[illegible]regulating IGF2. [illegible] findings might offer [illegible]tentia[illegible]erapeutic choice for patients wit[illegible]ian [illegible]er.

Key Word[illegible]

[illegible]ng no[illegible]A, DANCR, Ovarian cancer, [illegible]

## Introduction

[illegible]varian cancer is the second most prevalent and fatal gynecologic malignancy in the United States[1]. Due to unavailable recommendations for ovarian cance[illegible]ening, it [illegible]gnosed at an advance[illegible] This makes [illegible] of the leading cause[illegible] c[illegible]elated dea[illegible] in women. Appro[illegible]ately 22,[illegible] patients and 14,100 wo[illegible]ere diagnose[illegible]ied of ovarian cance[illegible] America in 2017, r[illegible]pectively[2]. Currently, th[illegible]ain treatment for patients with ovarian canc[illegible]cludes chem[illegible]rapy and surgery. However, [illegible]high rat[illegible] therapy resistance and metast[illegible]rs in almost 80% of women[3,4]. This s[illegible]ituation underscores the urgency [illegible] early detection and a new treatment for with [illegible]ancer.

[illegible] non-coding RNAs (lncRNAs) are a cluster of transcripts longer than 200 nucleotides without protein-coding function. Recent studies have revealed that lncRNAs are emerging with a variety of biological behaviors, including complex gene-regulatory networks in tumorigenesis. For example, lncRNA FAL1 functions as an oncogene in colon cancer by inhibiting cell apoptosis and promoting cell proliferation[5]. LncRNA-BANCR is positively associated with lymph node metastasis, differentiation and tumor staging of esophageal squamous cell carcinoma (ESCC). Meanwhile, lncRNA-BANCR is considered as a potential prognostic value for ESCC metastasis[6]. By regulating the expression of miR-335, lncRNA MSTO2P indirectly facilitates the proliferation and colony formation ability of gastric cancer cells[7]. LncRNA XIST, acting as an oncogene in bladder cancer, significantly promotes cell growth, migration and invasion by interacting with miR-124[8]. In addition, the overexpression of lncRNA GHET1 promotes the proliferation of pancreatic cancer. Furthermore, it is associated with TNM staging and prognosis of pancreatic cancer patients[9]. However, the exact role of lncRNA PANDAR in ovarian cancer and the possible underlying mechanism remain unclear so far.

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In our research, we found that the expression level of lncRNA DANCR in ovarian cancer tissues was significantly higher than the corresponding normal tissues. Moreover, DANCR promoted the proliferation, migration and invasion of ovarian cancer cells *in vitro*. Furthermore, our experiments explored the underlying molecular mechanism of DANCR in ovarian cancer progression.

## Patients and Methods

### *Clinical Samples and Cell Lines*

52 ovarian cancer patients who received surgery at Affiliated Huxi Hospital of Jining Medical College, Shanxian Central Hospital were enrolled in this study. Before the surgery, informed consent was obtained from each subject. This study was approved by the Research Ethics Committee of Affiliated Huxi Hospital of Jining Medical College, Shanxian Central Hospital. All collected tissues were confirmed by an experienced pathologist. Tissues were collected from the surgery and stored immediately at -80°C.

Four ovarian cancer cell lines (A2780, [illegible] V112D, OVCAR-3 and SKOV3) and one [illegible] ovarian cell line (ISOE80) were obtained fr[illegible] the Chinese Type Culture Collection, Chinese [illegible]emy of Sciences (Shanghai, China). All cells [illegible] cultured in Dulbecco's Modifie[illegible]le's Me[illegible]um (DMEM, Hyclone, South [illegible]T, USA) containing 10% fetal bovi[illegible]um ([illegible]; Gibco, Grand Island, NY, USA) [illegible] peni[illegible] [illegible]while, all cells were m[illegible]inta[illegible]% C[illegible]d 37°C humidified in[illegible]or.

### *Cell Transfe[illegible]*

The pLent[illegible]1a-[illegible]2A-Puro vector was used for [illegible]ing lentivi[illegible] targeting DANCR (Bi[illegible]a Inc., San Die[illegible]A, USA). DANCR le[illegible]ruses [illegible]ANCR) and empty vector were fir[illegible]age[illegible] 293T cells. Subsequently, they were [illegible]d into [illegible]ian cancer cells. DANCR expr[illegible] in t[illegible]ected cells was detected [illegible]antita[illegible]ime-Polymerase Chain Reac[illegible]n (qRT-PC[illegible] after 48 h.

### *[illegible]ion and qRT-PCR*

Total RNA in tissues and cells was extracted [illegible]ding to the instructions of TRIzol reagent ([illegible]ogen, Carlsbad, CA, USA). Extracted RNA was then reverse-transcribed to complementary deoxyribose nucleic acids (cDNAs) in strict accordance with the Reverse Transcription Kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). Primers used in this study were as follows: DANCR, forwards: 5′-CCTATCCCTTTCTCTAAGAA-3′ and reverse: 5′-ACTTCTGCAAA[illegible]AGGTGCTG-3′; Insulin-like growth [illegible] (IGF2), forward: 5′-TACCGGACTC[illegible]ATCTCGAGATGGCCGCCAACATGTA[illegible]-3′ and reverse: 5′-GATCCCGGGCCCG[illegible]ACCGTGTCCTCGATGACGATGG[illegible]TC-3[illegible]eraldehyde 3-phosphate deh[illegible]genase ([illegible]) forward: 5′-CCAAA[illegible]AGAT[illegible]GGGC[illegible]GCTGG-3′ and re[illegible] 5′-[illegible]TGGCA[illegible]GACTGTGGTCATTC[illegible]al cyc[illegible]was as follows: 95°C fo[illegible]sec, [illegible]r 40 [illegible]es and 60°C for 35 se[illegible]

### *Colony [illegible]ssay*

5×10[illegible]ells were [illegible] with FBS in a 6-well plat[illegible]12 days. Afte[illegible]he cells were fixed wi[illegible]ethanol and staine[illegible]ith 0.1% crystal viole[illegible]nally, the number of surviving colonies (>50 c[illegible]er colony) w[illegible]ounted and compared.

### *Ce[illegible]n Assay*

The [illegible] of transfected cells in 96-well [illegible]ates was monitored every 24 h according to the [illegible]ions of the Cell Counting Kit-8 (CCK-8) ([illegible]ojindo Molecular Technologies, Inc., Ku[illegible]amoto, Japan). The absorbance at 450 nm was detected *via* spectrophotometer (Thermo Scientific, Waltham, MA, USA). Three independent experiments were performed.

### *Transwell Assay*

For cell migration, 5 $\times 10^4$ cells in 200 µL of serum-free DMEM were seeded into the upper chamber of an 8 µm pore size insert (Millipore, Billerica, MA, USA). For cell invasion, the upper chamber was first coated with 50 µg Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). The bottom chamber was added with DMEM and FBS. 48 h later, the top surface of chambers was wiped by cotton swab and immersed with precooling methanol for 10 min. Then, the cells were stained with crystal violet for 30 min. Three fields were randomly selected, and the number of migrated and invaded cells was counted.

### *Western Blot Analysis*

Radio-immunoprecipitation assay (RIPA; Beyotime, Shanghai, China) was utilized to extract total protein from cells. The concentration of extracted protein was detected by the bicinchoninic acid (BCA) protein assay kit (TaKaRa, Otsu,

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Shiga, Japan). Target proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Then, the membranes were incubated with primary and secondary antibodies. Rabbit anti-GAPDH (glyceraldehyde 3-phosphate dehydrogenase) and rabbit anti-IGF2, as well as goat anti-rabbit secondary antibody were provided by Cell Signaling Technology (CST, Danvers, MA, USA). The chemiluminescent film was applied for assessment of the protein expression with Image J software (NIH, Bethesda, MD, USA).

### Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Data were presented as mean ± SD (standard deviation). The Student *t*-test and Chi-square test were conducted appropriately. $p<0.05$ was considered statistically significant.

## Results

### Expression Level of DANCR in Ovarian Cancer Tissues and Cells

First, the expression level of [illegible]NCR in [illegible] ovarian tissue samples and 4 [illegible]ncer ce[illegible] lines was detected by qRT[illegible]R. T[illegible] result revealed that DANCR was [illegible]antl[illegible]d in ovarian cancer tissu[illegible] (Fi[illegible] Mea[illegible], DANCR expression in ovarian cancer cells was remarkably higher than that of normal ovarian cell line ISOE80 (Figure 1B).

### Overexpression of DANCR Prom[illegible] the Proliferation of Ovarian Ca[illegible] Cells

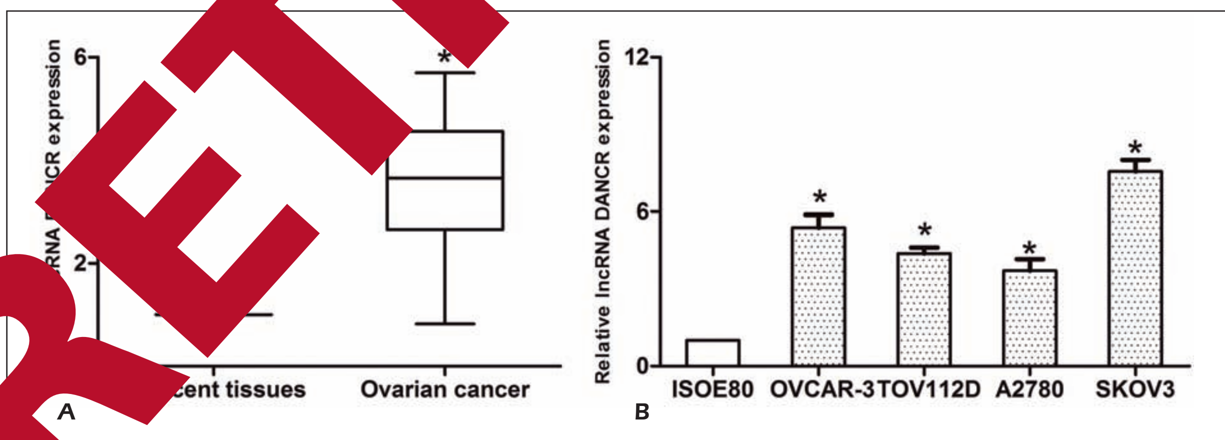
Ovarian cancer cell line A2780 [illegible] chosen for DANCR overexpression in this stu[illegible]NCR expression in transfected cells w[illegible] dete[illegible] qRT-PCR (Figure 2A). Colony f[illegible]tion assay [illegible] that the overexpression [illegible]DANC[illegible] signi[illegible] promoted ovarian can[illegible]ll gr[illegible] (Figure 2[illegible]). Moreover, cell prolife[illegible] indic[illegible]d that the growth abilit[illegible] ovar[illegible]cer c[illegible] was facilitated after D[illegible]CR overex[illegible]gure 2C).

### Overexp[illegible]sio[illegible] DANCR Enha[illegible]d Migra[illegible]nd Invasion of O[illegible]an Cancer

[illegible]und healing assay [illegible]monstrated that after D[illegible]CR overexpression, the migration ability of o[illegible]n cancer cel[illegible] was significantly promoted (F[illegible] 3A). Fur[illegible]hore, transwell assay also indica[illegible]mber of migrated and invaded cells w[illegible]rkably increased in ovarian cancer [illegible]lls overexpressing DANCR (Figure 3B and 3C).

### [illegible]tion Between IGF2 and DANCR in Ovarian Cancer

Numerous studies have revealed IGF2 acts as an oncogene in multiple cancers, including ovarian cancer. Subsequently, we conducted mechanism assays to explore the interaction between ROCK1 and PANDAR. The results of qRT-PCR revealed that the




[illegible]1. The expression of DANCR was significantly increased in ovarian cancer tissues and cell lines, which was asso[illegible]d with worse overall survival of ovarian cancer patients. **A**, DANCR expression was markedly increased in ovarian cancer tissues compared with adjacent tissues. **B**, The expression level of DANCR relative to GAPDH was determined in human ovarian cancer cell lines and normal ovarian cell line ISOE80 by qRT-PCR. Data were presented as mean ± standard error of mean. $*p<0.05$.

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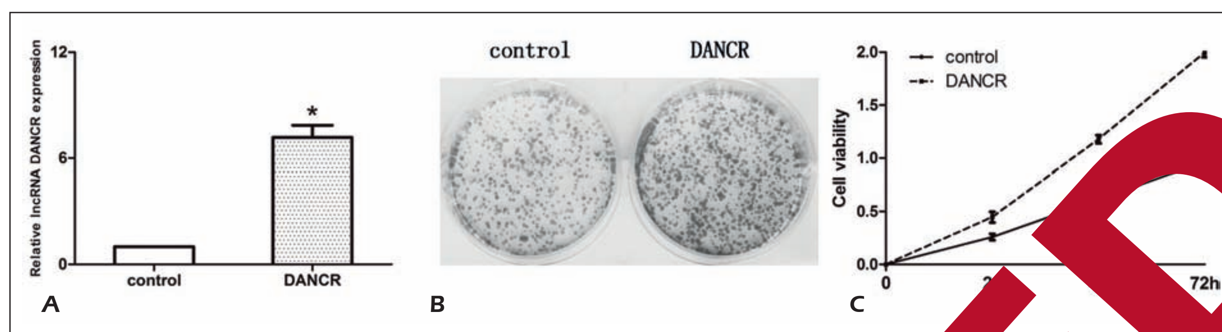


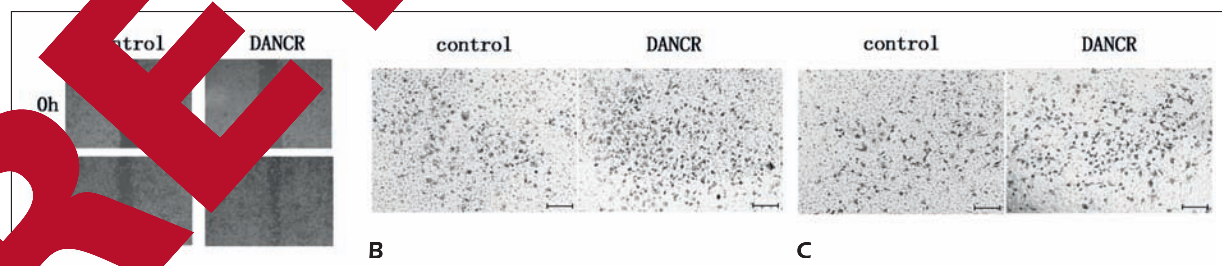

**Figure 2.** Overexpression of DANCR promoted ovarian cancer cell proliferation. **A**, DAN[illegible]pressi[illegible]ovarian ca[illegible]r cells transduced with DANCR lentiviruses (DANCR) and empty vector (control) was dete[illegible]R. GA[illegible]DH was used as an internal control. **B**, Colony formation assay showed that the number of colo[illegible] in [illegible]ntiviru[illegible]p was remarkably increased compared with the empty control group. **C**, Cell proliferation [illegible] showed [illegible]ove[illegible]ssion of DANCR significantly increased the growth of ovarian cancer cells. The results re[illegible]ed the averag[illegible]dependent experiments (mean ± standard error of the mean). *$p<0.05$, as compared with t[illegible]ells.

expression level of IGF2 in cells of DANCR lentiviruses (DANCR) group was significantly higher than that of the cells in the empty vector (control) group (Figure 4A). Western blot assay showed that after DANCR overexpression, the protein expression of IGF2 was significantly upregulated (Figure 4B). Furthermore, we found out that the expressi[illegible]f IGF2 in ovarian cancer tissues was marked[illegible]er when compared with the adjacent tissues [illegible]re 4C). The correlation analysis demonstrated [illegible]t IGF2 expression level was positively correlated [illegible] DANCR expression in cancer tiss[illegible]gure 4D).

## Dis[illegible]ssi[illegible]

Evidence has [illegible]d that lnc[illegible]are crucial factors in t[illegible]ment of ova[illegible] cancer. Meanwhile, l[illegible]NA[illegible]e used as potential indi[illegible]s of ovarian [illegible]or even classifiers fo[illegible]sonalized therapy. [illegible]r instance, the prolif-e[illegible]n and migration of ovarian cancer cells are s[illegible]cantly inhi[illegible]d after knockdown of lnc[illegible]MNX1-A[illegible]. This indicates that lncRNA M[illegible]serve as a potential target for ovaria[illegible]r[10]. LncRNA CCAT1 promotes [illegible]ll epithelial-mesenchymal transition, migra-[illegible]invasion of epithelial ovarian cancer[11]. [illegible] linc01234 regulates the proliferation, invasion and apoptosis of esophageal cancer cells[12]. Moreover, downregulation of lncRNA PCA3 significantly suppresses the proliferation, invasion and migration of epithelial ovarian cancer cells[13].

LncRNA DANCR is a newly discovered oncogenic lncRNA. DANCR promotes the proliferation and tumorigenesis of gastric cancer, acting as a potential prognostic indicator and therapeutic target[14]. DANCR promotes HM-




[illegible]e 3. Overexpression of DANCR promoted ovarian cancer cell migration and invasion. **A**, Wound healing assay showed t[illegible]migration of ovarian cancer cells was markedly increased after overexpression of DANCR in ovarian cancer cells. **B**, The[illegible]nswell assay showed that the number of migrating cells was significantly increased after overexpression of DANCR in ovarian cancer cells (magnification, 40X). **C**, The transwell assay showed that the number of invading cells was remarkably increased after overexpression of DANCR in ovarian cancer cells (magnification, 40X). The results represented the average of three independent experiments (mean ± standard error of the mean). *$p<0.05$, as compared with the control cells.

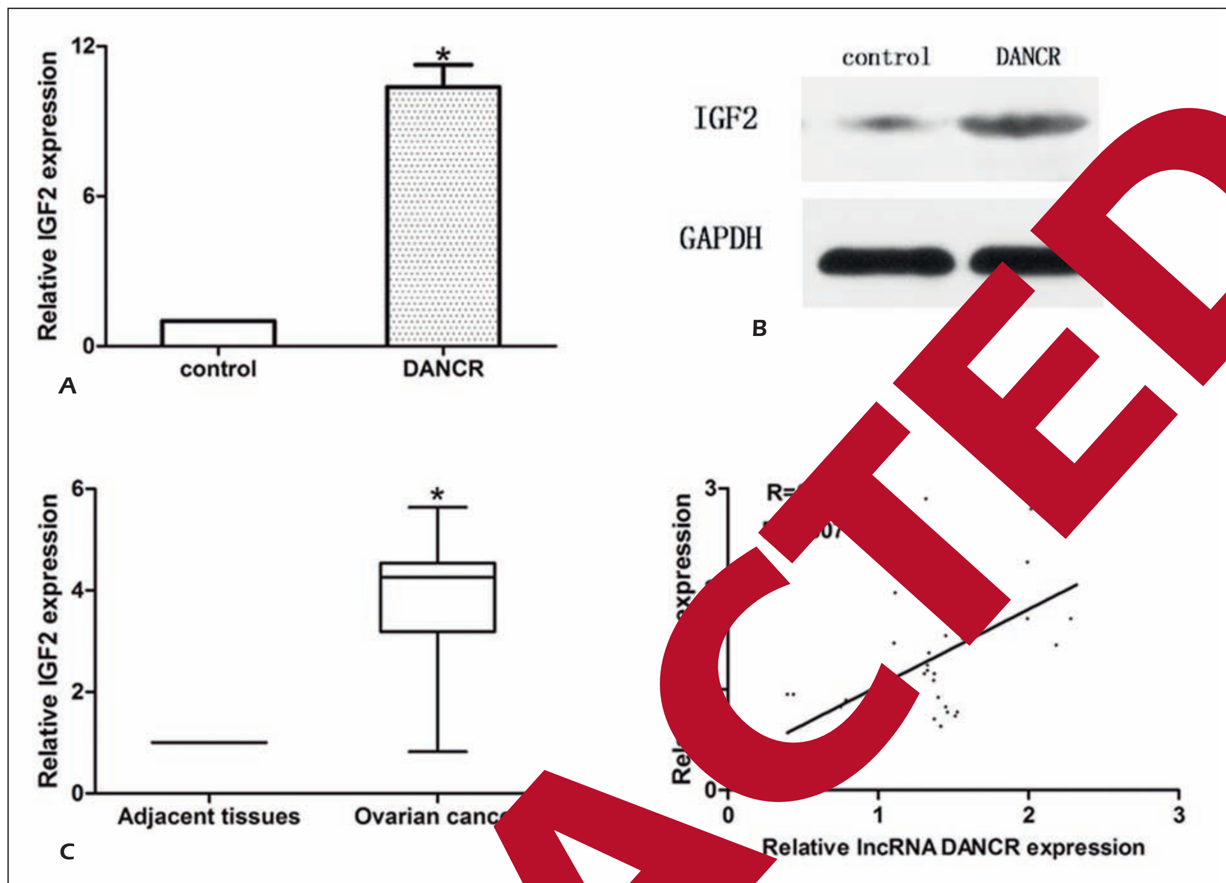



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**Figure 4.** Interaction between DANCR and IGF2. [illegible]R results showed that IGF2 expression was significantly higher in DANCR lentiviruses (DANC[illegible]pared wit[illegible]mpty vector (control). **B**, Western blot assay revealed that the protein expression of was markedly [illegible] DANCR [illegible]iruses (DANCR) compared with the empty vector (control). **C**, IGF2 was remarkably upregula[illegible] ova[illegible]cancer tiss[illegible]mpared with adjacent tissues. **D**, Linear correlation between the expression level of IGF2 an[illegible]NCR in[illegible]ancer [illegible]sues. The results represented the average of three independent experiments. Data were presen[illegible]rror of the mean. *$p<0.05$.

GA2-mediated [illegible] of lung ad[illegible]carcinoma cells[15]. The[illegible]vere[illegible]on of DANCR is related to th[illegible]dvanced p[illegible]ion of colorectal cancer, [illegible]ch may be a po[illegible] biomarker for progn[illegible]. Mo[illegible]ver, DANCR plays a crucial rol[illegible]cre[illegible]g the stemness features of hepatoc[illegible]cinom[illegible] inhibiting CTNNB1[17]. [illegible]this w[illegible] fo[illegible] that DANCR was highly [illegible]ssed i[illegible]rian cancer tissues and cell li[illegible] Besides, [illegible] DANCR overexpression, the p[illegible] migration and invasion abilities of [illegible]r cells were significantly promot[illegible] The above results indicated that DANCR [illegible]ted tumorigenesis of ovarian cancer and m[illegible]act as an oncogene.

Insulin-like growth factor 2 (IGF2) is a protein hormone, which plays a crucial role in the regulation of cell proliferation, migration and differentiation[18]. The mRNA expression of IGF2 is markedly upregulated in fibroadenomas, especially in stromal cells[19]. IGF2 is overexpressed in colorectal cancer, which is remarkably correlated with the sensitivity of colorectal cancer to IGF1R/INSR inhibitor BI 885578[20]. Highly expressed IGF2 participates in angiogenesis of invasive bladder cancer, which can also predict poor prognosis of patient[21]. Moreover, the upregulation of IGF2 is remarkably related to poor prognosis of ovarian cancer[22]. In our research, IGF2 expression was significantly upregulated after overexpression of DANCR. Moreover, IGF2 expression in ovarian cancer samples was positively correlated with DANCR expression. All the results above suggested that DANCR might promote tumorigenesis of ovarian cancer *via* upregulating IGF2.

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## Conclusions

We identified that DANCR expression was remarkably upregulated in patients with ovarian cancer. Besides, DANCR could enhance the proliferation, migration and invasion of ovarian cancer cells by upregulating IGF2. These findings suggested that DANCR might contribute to therapy for ovarian cancer as a candidate target.

### Conflict of Interests

The authors declare that they have no conflict of interest.

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