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LncRNA DANCR aggravates the progression of ovarian cancer by downregulating UPF1

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Abstract. – OBJECTIVE: To uncover the role of long non-coding RNA (lncRNA) DANCR in aggravating the progression of ovarian cancer (OC) by downregulating UPF1 level.

PATIENTS AND METHODS: DANCR level in OC tissues and matched adjacent normal ones was determined by quantitative real-time polymerase chain reaction (qRT-PCR). Its expression level in OC patients with different tumor node metastasis (TNM) staging and either with metastasis, or not, was examined as well. Receiver operating characteristic (ROC) curves were introduced for assessing the prognostic value of DANCR in OC. Subsequently, regulatory effects of DANCR on proliferative and migratory abilities of HO8910 and HEY cells were evaluated. Subcellular distribution of DANCR in OC cells was analyzed. Furthermore, the interaction between DANCR and UPF1 was explored by RNA immunoprecipitation (RIP) and Pearson correlation analysis. Finally, rescue experiments were conducted to clarify the role of DANCR/UPF1 axis in the progression of OC.

RESULTS: DANCR was upregulated in OC tissues and cell lines. Its level was higher in OC patients with worse tumor stage and accompanied by metastatic loci. DANCR exerted the potential to serve as a prognostic marker for OC. Overexpression of DANCR accelerated HO8910 and HEY cells to proliferate and migrate. UPF1 was found to be downregulated in OC tissues and negatively correlated to DANCR. DANCR was mainly distributed in the cytoplasm and interacted with UPF1. Overexpression of UPF1 in OC cells partially reversed the promotive effect of DANCR on proliferative and migratory rates.

CONCLUSIONS: LncRNA DANCR accelerates the proliferative and migratory abilities of OC cells through negatively regulating UPF1 level, thus aggravating the progression of OC.

Key Words:
Ovarian cancer, DANCR, UPF1.

Introduction

Ovarian cancer (OC) is one of the most common malignant tumors in the female reproductive system. It is estimated that about 220,000 women are diagnosed with OC every year, and about 140,000 people die of OC, accounting for 2.3% of all tumor deaths[1]. The prognosis of advanced OC patients is poor[2]. Hence, improving the detective rate of OC as early as possible urgently needs to be solved. Currently, conventional detection methods for OC include gynecological examination, gynecological B-ultrasound, examinations of serum tumor markers (i.e., CA125, CA199), pelvic magnetic resonance and etc. Among them, determination of CA125 level and gynecological B-ultrasound have important significance in the differential diagnosis of OC. Nevertheless, the specificities of these examinations are not very high for early-stage OC[3]. It is necessary to develop novel diagnostic hallmarks for OC.

Long non-coding RNAs (LncRNAs) are defined as RNAs of at least 200 nt long. They are independently transcribed and have no potential to encode functional proteins. In recent years, whole-genome analysis has been well concerned, which pushes forward to lncRNA researches[4]. Differentially expressed lncRNAs in OC have been identified, which are capable of mediating the cellular behaviors of tumor cells[5]. Previous studies have clarified that lncRNA DANCR is involved in the progression of retinoblastoma[6] and glioma[7]. The specific function of DNACR in OC has not been fully investigated.

Nonsense-mediated mRNA decay (NMD) is a mRNA quality monitoring mechanism widely found in eukaryotic cells. NMD prevents the production of potentially toxic truncated proteins by identifying and degrading transcripts containing premature translational-termination codon (PTC)[8]. In tumor biology, NMD exerts a regulatory role by targeting mutant gene proteins[9,10]. However, NMD is downregulated under the circumstances of tumor cell nutrient deficiency and endoplasmic reticulum overload, suggesting the absence of NMD during tumorigenesis[10]. The occurrence of NMD is influenced by various polypeptides, such as UPF1, UPF2, UPF3, Y14, SMG1, SMG5, SMG6, and SMG7[11]. Up-frame shift mu-

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tant 1 (UPF1) is a key mediator in the NMD pathway[12]. Moreover, UPF1 level determines the NMD pathway[13]. This study mainly explored the role of DANCR/UPF1 in the progression of OC, which may provide new directions in the clinical treatment of OC.

Patients and Methods

Sample Collection

OC tissues (n=20) and adjacent normal tissues were surgically harvested from OC patients admitted in Xiangya Hospital Central South University from April 2016 to December 2018. TNM staging and metastatic statue of enrolled OC patients were recorded. None of these patients were preoperatively treated. This study was approved by the Medical Ethic Committee of Xiangya Hospital Central South University and the written informed consent from each subject was obtained.

Cell Culture and Transfection

Epithelial ovarian cell line (IOSE-386) and OC cell lines (SKOV-3, OVCAR3, HO8910, and HEY) were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Cell culture was conducted in Roswell Park Memorial Institute-1640 (RPMI-1640; HyClone, South Logan, UT, USA) with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) in an incubator with 5% CO_2 at 37°C. For cell transfection, 1.5 ml of serum-free medium and 0.5 mL of LipofectamineTM 2000 (Invitrogen, Carlsbad, CA, USA) containing transfection vectors were applied in each well of a 6-well plate. Fresh medium was replaced 4-6 h later. Transfected cells for 24-48 h were collected for determination.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA in cells and tissues was extracted using TRIzol method (Invitrogen, Carlsbad, CA, USA) for reverse transcription according to the instructions of PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). RNA concentration was detected using a spectrometer. QRT-PCR was then performed based on the instructions of SYBR Premix Ex Taq TM (TaKaRa, Otsu, Shiga, Japan). Primer sequences were as follows: DANCR-F: 5'-GCCACTATGTAGCGGGTTTC-3', DANCR-R: 5'-GCCTGTAGTTGTCAACCTGC-3'; UPF1-F: 5'-GTCAGATCGCCTGGAGAATT-3', UPF1-R: 5'-AGTATTTCCACGTCCGTTGC-3'; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)-F: 5'-CAATGACCCCTTCATTGACC-3', GAPDH-R: 5'-GACAAGCTTCCCGTTCTCAG-3'.

Cell Counting Kit-8 (CCK-8) Assay

Transfected cells were seeded into 96-well plates at a density of 2×10^3 cells/100 μL. At the appointed time points, 10 μL of CCK-8 solution (Dojindo Molecular Technologies, Kumamoto, Japan) was added in each well. The absorbance at 450 nm of each sample was measured by a microplate reader (Bio-Rad, Hercules, CA, USA).

Transwell Migration Assay

Transfected cells for 48 h were adjusted to the dose of 1.0×10^5 cells/mL and subjected to serum starvation for 12 h. 200 μL/well suspension was applied in the upper side of transwell chamber (Millipore, Billerica, MA, USA). In the bottom side, 700 μL of medium containing 10% FBS was applied. After 48 h of incubation, cells migrated to the bottom side were subjected to fixation in methanol for 15 min, crystal violet staining for 20 min and cell counting using a microscope. Penetrating cells were counted in 5 randomly selected fields per sample.

RNA Immunoprecipitation (RIP)

Cells were treated according to the procedures of Millipore Magna RIPTM RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA). Cell lysate was incubated with input, anti-UPF1, or anti-IgG antibody at 4°C for 6 h. A protein-RNA complex was captured and digested with 0.5 mg/mL proteinase K containing 0.1% SDS to extract RNA. The magnetic beads were repeatedly washed with RIP washing buffer to remove non-specific adsorption as much as possible. Finally, the extracted RNA was subjected to mRNA level determination using qRT-PCR.

Determination of Subcellular Distribution

Cytoplasmic and nuclear RNAs were extracted using the PARIS kit (Invitrogen, Carlsbad, CA, USA) and subjected to qRT-PCR. U6 was the internal reference of nucleus and GAPDH was that of cytoplasm.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 statistical software (IBM, Armonk, NY, USA) was used for data analysis. Data were expressed as mean ± standard deviation ($\bar{x}\pm s$). Intergroup data were compared using the *t*-test.

Receiver operating characteristic (ROC) curves were introduced for assessing the prognostic potential of DANCR in OC. Pearson correlation analysis was conducted for evaluating the relationship between expression levels of DANCR and UPF1. $p<0.05$ considered the difference was statistically significant.

Results

Upregulated DANCR in OC

QRT-PCR data showed higher abundance of DANCR in OC tissues relative to matched normal ones (Figure 1A). Its level in OC patients increased with the worsen of TNM staging. DANCR level remained higher in OC patients with stage III-IV than those with stage I-II (Figure 1B). Moreover, metastatic OC patients presented higher level of DANCR than those without metastatic loci (Figure 1C). ROC curves indicated the potential of DANCR as a prognostic marker for OC (AUC=0.852, cut-off value=1.354, Figure 1D).

DANCR Accelerated the Proliferative and Migratory Abilities of OC

DANCR was identically upregulated in OC cells, especially in HEY and HO8910 cells (Figure 2A). Transfection of pcDNA-DANCR in these two cell lines markedly upregulated DANCR level, showing a pronounced transfection efficacy (Figure 2B). After transfection of pcDNA-DANCR, CCK-8 assay showed the elevated viability in HO8910 and HEY cells (Figure 2C, 2D). Besides, transwell assay showed the increased number of migratory cells in HO8910 and HEY cells overexpressing DANCR, suggesting the elevated migratory ability (Figure 2E). It is concluded that DANCR markedly promoted the proliferative and migratory rates of OC cells.

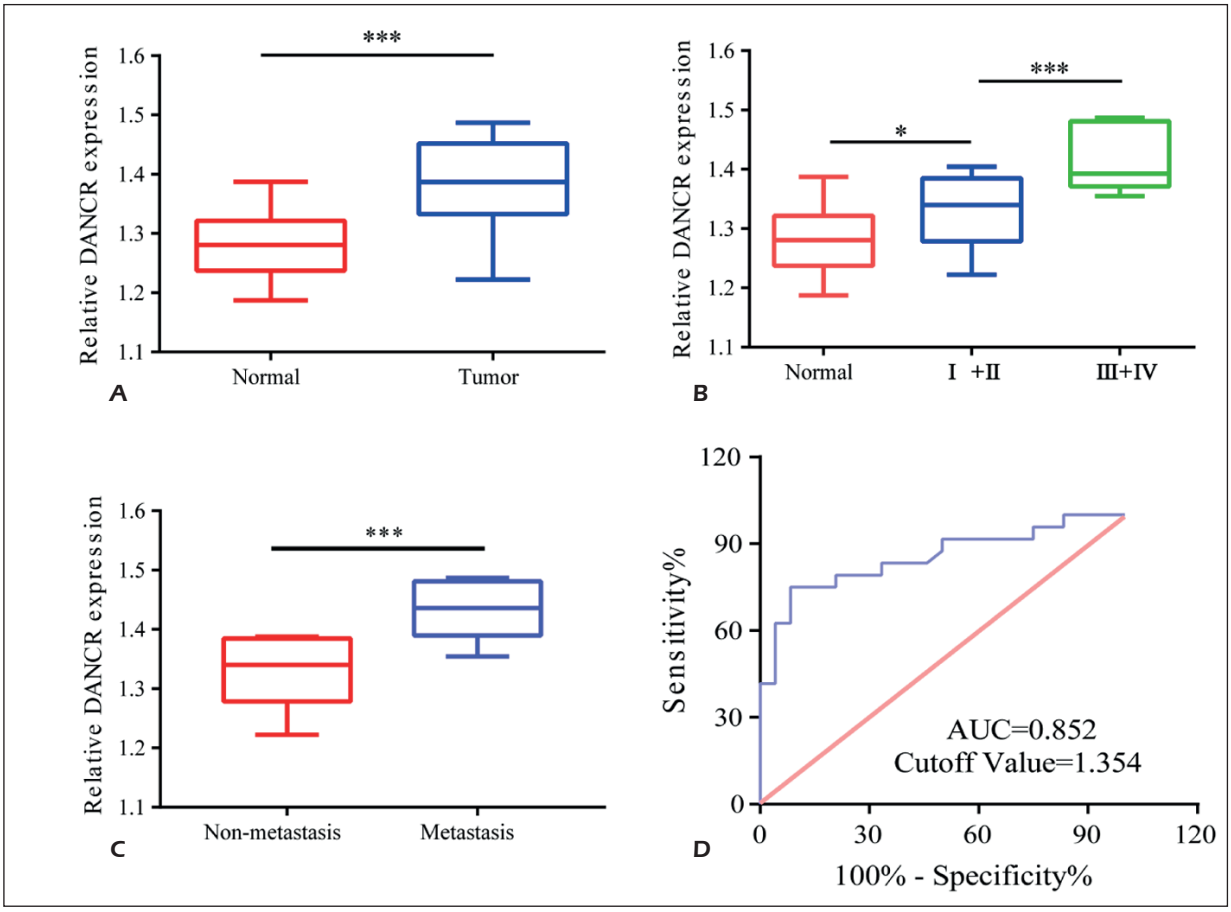

Figure 1. Upregulated DANCR in OC. **A**, Relative level of DANCR in OC tissues and normal ovarian tissues. **B**, Relative level of DANCR in normal ovarian tissues, OC with stage I-II and stage III-IV. **C**, Relative level of DANCR in OC patients either with metastasis or not. **D**, ROC curves introduced for assessing the sensitivity and specificity of DANCR in diagnosing OC (AUC=0.852, cut-off value=1.354).

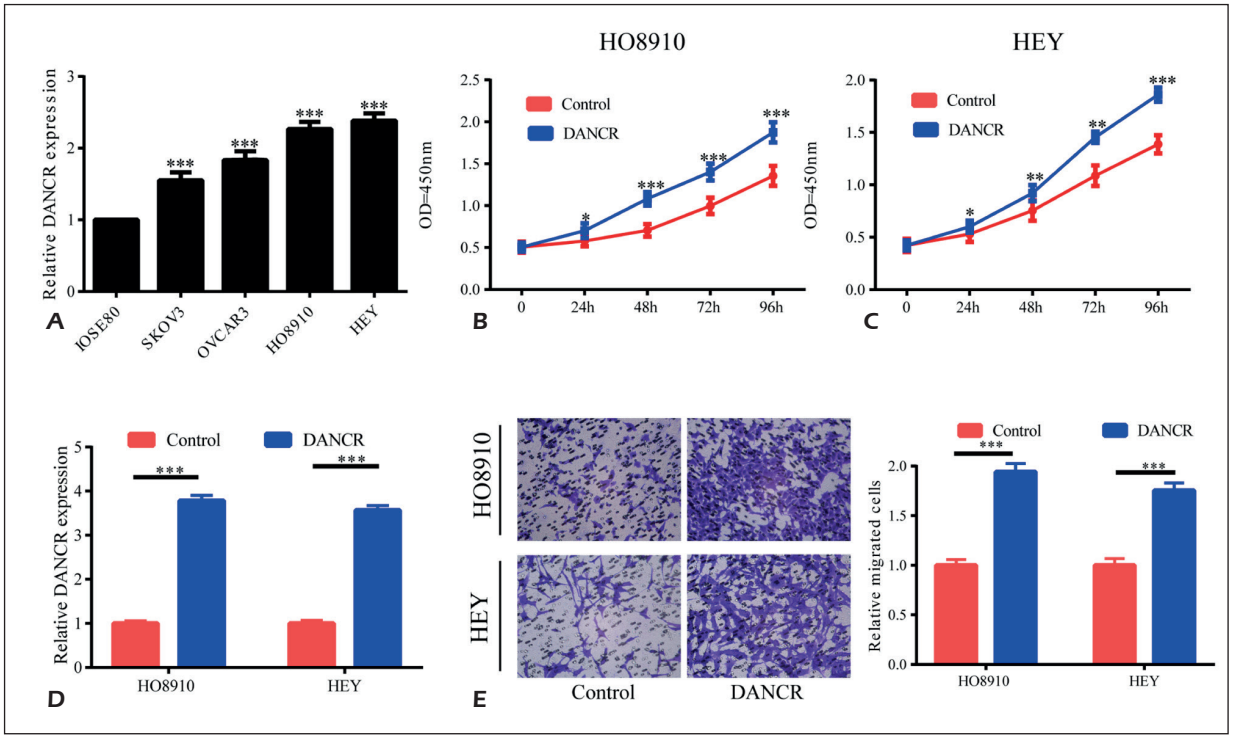

Figure 2. DANCR accelerated the proliferative and migratory abilities of OC. **A**, Relative level of DANCR in normal ovarian epithelial cell line and OC cell lines. **B**, Transfection efficacy of pcDNA-DANCR in HO8910 and HEY cells. **C**, CCK-8 assay indicated the viability in HO8910 cells transfected with negative control or pcDNA-DANCR. **D**, CCK-8 assay revealed the viability in HEY cells transfected with negative control or pcDNA-DANCR. **E**, Transwell assay detected migratory cell number in HO8910 and HEY cells transfected with negative control or pcDNA-DANCR (magnification: 40×).

DANCR Interacted with UPF1 and Downregulated Its Level

UPF1 level was found to be downregulated in OC tissues than that of controls (Figure 3A). Subsequently, a negative correlation between expression levels of DANCR and UPF1 was identified in OC tissues (R^2=0.2656, p=0.010) (Figure 3B). Transfection of pcDNA-DANCR remarkably downregulated UPF1 level in HO8910 and HEY cells, further proving their negative correlation (Figure 3C). It is speculated that UPF1 may be interacted with DANCR to participate in the progression of OC. As RIP assay revealed, higher enrichment of DANCR was observed in anti-UPF1 compared with that of anti-IgG, demonstrating the interaction between DANCR and UPF1 (Figure 3D). Moreover, subcellular distribution analysis suggested that DANCR was mainly expressed in cytoplasm of OC cells (Figure 3E, 3F).

DANCR Mediated the Progression of OC Via Targeting UPF1

We constructed pcDNA-UPF1 and tested its transfection efficacy in OC cells (Figure 4A). Notably, the elevated viability in HO8910 and HEY cells overexpressing DANCR was partially reversed after co-transfection of pcDNA-UPF1 (Figure 4B, 4C). Similarly, the increased number of migratory cells by overexpression of DANCR was reversed after co-transfection of pcDNA-UPF1 (Figure 4D). Hence, we believed that DANCR promoted the proliferative and migratory rates of OC cells *via* negatively regulating UPF1.

Discussion

OC is a prevalent gynecologic tumor. Many OC patients are diagnosed in advanced stage owing to the atypical symptoms and rapid progression. The mortality of OC ranks the first in gynecological tumors, and its 5-year survival is about 30%[14]. The pathogenesis of OC is a complex biological process, involving both genetic and epigenetic changes[15]. So far, several genes that closely related to the progression of OC have been identified, including BRCA1/2, p53, and K-Ras[15-17]. It is of great significance to uncover the pathogenesis of OC, so as to improve the prognosis of OC patients.

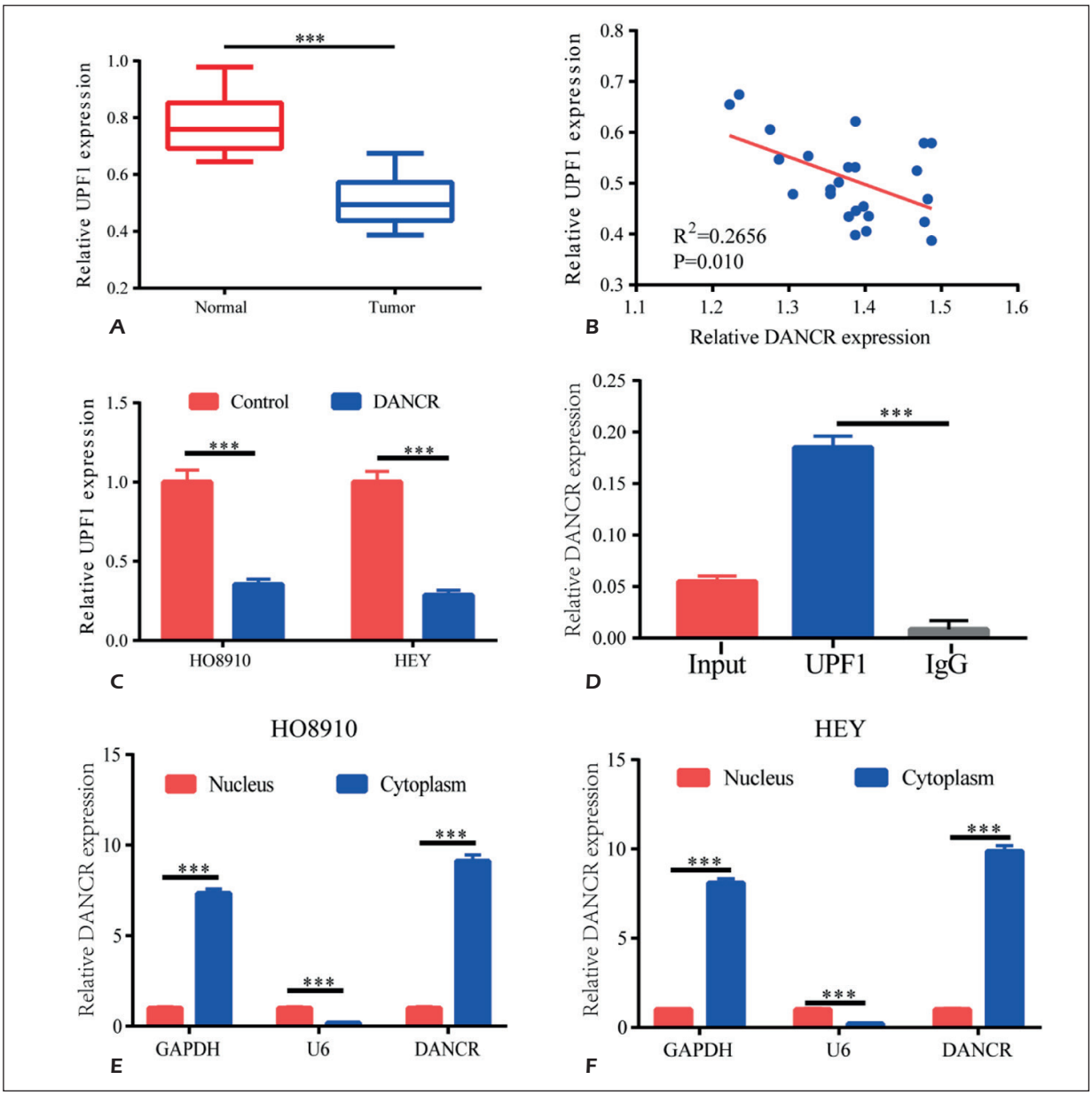

Figure 3. DANCR interacted with UPF1 and downregulated its level. **A**, Relative level of UPF1 in OC tissues and normal ovarian tissues. **B**, A negative correlation between expression levels of DANCR and UPF1 in OC tissues (R^2=0.2656, p=0.010). **C**, Relative level of UPF1 in HO8910 and HEY cells transfected with negative control or pcDNA-DANCR. **D**, RIP assay showed the enrichment of DANCR in input, anti-UPF1 and anti-IgG. **E**, Subcellular distribution of DANCR in nucleus and cytoplasm of HO8910 cells. GAPDH and U6 were served as cytoplasmic and nuclear internal reference, respectively. **F**, Subcellular distribution of DANCR in nucleus and cytoplasm of HEY cells. GAPDH and U6 were served as cytoplasmic and nuclear internal reference, respectively.

LncRNAs are the member of the non-coding RNA family. They were once considered to be the "noise" of genomic transcripts. However, as the research progresses, it is found that lncRNA is involved in various biological processes, in different regulatory mechanisms[18]. Basically, there are three regulatory mechanisms of lncRNAs as Matthieu et al[19] reported. First of all, lncRNAs regulate gene expressions at transcriptional and translational levels. They localize the protein complexes to the corresponding DNA sequences after binding to the proteins. Meanwhile, they are able to bind to multiple transcriptional factors as a central platform to regulate downstream genes. Secondly, lncRNAs serve as ceRNAs to influence RNA functions. Thirdly, lncRNAs could be used

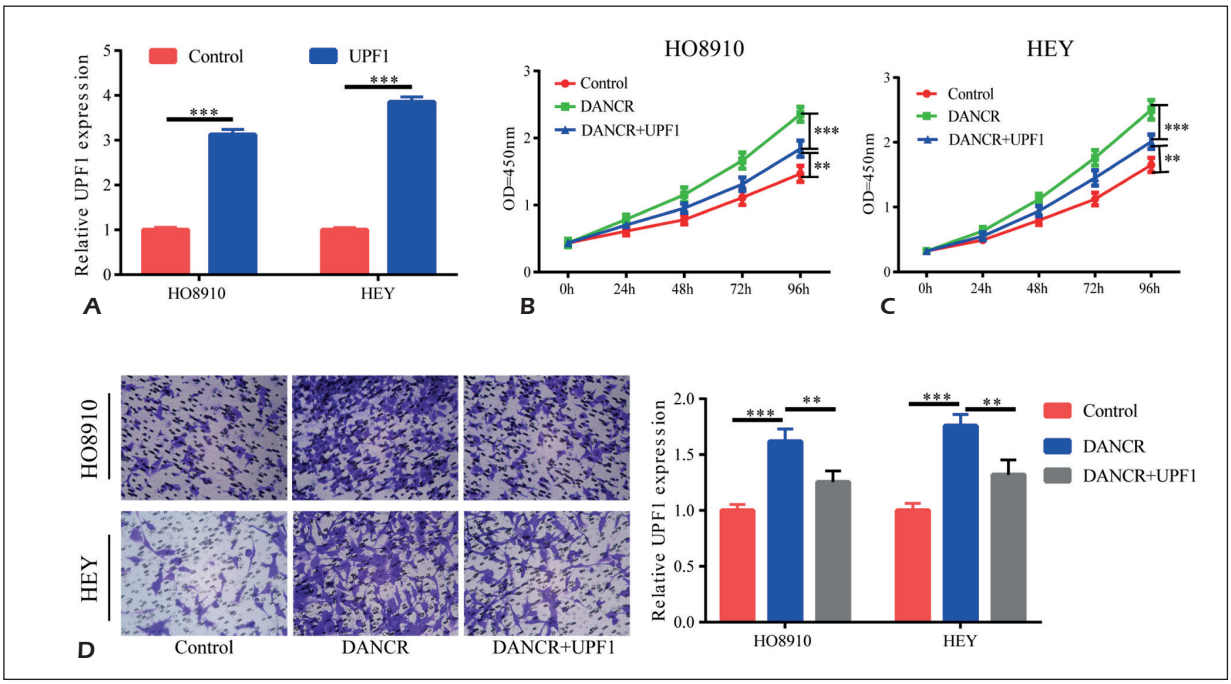

Figure 4. DANCR mediated the progression of OC via targeting UPF1. **A**, Transfection efficacy of pcDNA-UPF1 in HO8910 and HEY cells. **B**, CCK-8 assay showed the viability in HO8910 cells transfected with negative control, pcDNA-DANCR or pcDNA-DANCR+pcDNA-UPF1. **C**, CCK-8 assay showed the viability in HEY cells transfected with negative control, pcDNA-DANCR or pcDNA-DANCR+pcDNA-UPF1. **D**, Transwell assay showed migratory cell number in HO8910 and HEY cells transfected with negative control, pcDNA-DANCR or pcDNA-DANCR+pcDNA-UPF1 (magnification: 40×).

as miRNA precursors. The above three mechanisms are interactive in regulating biological functions[20].

In recent years, lncRNAs have shown a promising aspect in developing drug targets on clinical treatment of ovarian epithelial cancer. For example, H19 mediates cisplatin-resistance in high-grade serous OC by regulating glutathione metabolism[21]. UCA1 exerts a crucial role in the metastasis of ovarian epithelial cancer[22]. HOXA11AS accelerates the invasion of OC through targeting VEGF and MMP-9[23]. Gao et al[24] reported that HOST2 is upregulated in OC as an oncogene that affects the proliferative rate of cancer cells. Consistently, we showed upregulation of DANCR in OC tissues, indicating a potential effect on the progression of OC. Overexpression of DANCR markedly accelerated the proliferative and migratory abilities of OC cells. In addition, DANCR was verified to interact with UPF1 and negatively regulated its level. Notably, UPF1 overexpression reversed the promotive effect of DANCR on the proliferative and migratory rates of OC cells. Collectively, DANCR served as a carcinogenic gene in OC by downregulating UPF1 level.

Conclusions

We found that lncRNA DANCR accelerates the proliferative and migratory abilities of OC cells through negatively regulating UPF1 level, thus aggravating the progression of OC.

Conflict of Interests

The authors declare that there are no conflicts of interest.

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