LncRNA DLGAP1-AS2 Suppresses the Maturation of miR-16 to Suppress Cell Invasion and Migration of Ovarian Cancer Cells

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Research Article

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Abstract

Background: This study aimed to explore the role of lncRNA DLGAP1-AS2 in ovarian cancer (OC).

Methods: Expression of DLGAP1-AS2, mature miR-16 and miR-16 precursor in paired OC tissues and non-tumor tissues collected from 62 OC patients was determined by RT-qPCR. Correlations were analyzed by Pearson's correlation coefficient. Overexpression of DLGAP1-AS2 was achieved in OC cell line UWB1.289 to explore the effects of DLGAP1-AS2 overexpression on the expression of mature miR-16 and miR-16 precursor by RT-qPCR. Transwell assays were performed to analyze the role of DLGAP1-AS2 and miR-16 in regulating the invasion and migration of UWB1.289 cells.

Results: DLGAP1-AS2 was upregulated in OC and inversely correlated with mature miR-16, but not miR-16 precursor. In OC cells, DLGAP1-AS2 overexpression resulted in downregulated mature miR-16, but failed to significantly affect the expression of miR-16 precursor. Cell invasion and migration analysis showed that DLGAP1-AS2 overexpression reduced the inhibitory effects of miR-16 overexpression on cell invasion and migration.

Conclusions: DLGAP1-AS2 may suppress the maturation of miR-16 to suppress cell invasion and migration of OC cells.

Introduction

Ovarian cancer (OC) is one of the most commonly diagnosed female malignancies and it mainly affects females between 50 and 70 years old [1]. It is estimated that 1 out of 70 females will eventually develop OC during their whole life time [2], and 1 out 108 108 females will die of this disease [3]. More than 80% of OC patients diagnosed at stage I can survive more than 5 years [4]. However, tumor metastasis distant sites, such as liver, the lungs, the intestines, the spleen, the brain and skin, is common in OC patients [5, 6]. Once distant metastasis has occurred, the overall 5-year survival rate will drop to below 20%, mainly owing to the lacking of effective treatments for tumor metastasis [5, 6]. Therefore, novel therapeutic approaches are needed.

Previous studies on the molecular mechanism of OC have characterized multiple molecular pathways involved in the growth and metastasis of this malignancy [7, 8]. In effect, some molecular players with crucial functions in OC have been proven to be potential targets for the development of novel targeted therapies that can be applied to suppress tumor growth or metastasis by regulating related gene expression network [9, 10]. However, effective targets for the treatment of OC remain lacking. LncRNAs have no direct role in protein synthesis, while they participate in human cancers, including OC by regulating gene expression [11]. Therefore, lncRNAs are proming targets for targeted therapy. Recently, the interactions between lncRNAs and other ncRNAs, such as miRNAs, have also been reported [12]. In a recent study DLGAP1-AS2 has been reported to play oncogenic roles in glioma [13]. However, the role of DLGAP1-AS2 in other cancers is unknown. We performed deep sequencing analysis and observed the

inverse correlation between DLGAP1-AS2 and miR-16, which is also a crucial player in cancer biology. This study was therefore carried out to explore the interactions between DLGAP1-AS2 and miR-16 in OC.

Materials And Methods

Tissue collections

Fine needle aspiration was performed on 62 OC patients (48 to 68 years old; 57.8+/-5.8 years) who were admitted to The First Affiliated Hospital, School of Clinical Medicine of Guangdong Pharmaceutical University, between January 2019 and May 2020. All tissue samples were confirmed by histopathological analysis, followed by storage in liquid nitrogen before the subsequent experiments. All the 62 OC patients were newly diagnosed cases and no therapy was initiated. No recurrent OC cases were included and other clinical disorders were excluded. The 62 OC patients included 28 cases at AJCC stage I or II and 34 cases at stage III or IV. The aforementioned hospital Ethics Committee approved this study. Written informed consent was provided by all patients.

UWB1.289 OC cells and transient transfections

Human OC cell line UWB1.289 from ATCC (USA) was used as the cell model of OC. RPMI-1640 medium was supplemented with 10% FBS to serve as the culture medium of UWB1.289 cells. A 5% CO_2 incubator was used to cultivate cells at 37 °C and 95% humidity. Cells used in the subsequent assays were collected at about 80% confluence.

Expression vector of DLGAP1-AS2 was established using pcDNA3.1 (Sigma-Aldrich). Mimic of miR-16 and negative control (NC) miR-16 were purchased from Invitrogen. UWB1.289 cells were transfected with 1 μg DLGAP1-AS2 expression vector or 40 nM miRNA through transient transfections mediated by lipofectamine 2000 (Invitrogen). To perform NC experiments, the same number of UWB1.289 cells were transfected with the same amount of empty vector or NC miRNA. In all transfections, control (C) cells were cells without transfections. After transfections, cells were cultivated in fresh cell culture medium for 48 h before the subsequent experiments.

RNA extractions

Isolation of RNA from paired tissues and UWB1.289 cells was performed by Ribozol (Invitrogen). Incubation with DNase I (Invitrogen) was performed for 100 min at 37 °C to achieve complete genomic DNA removal. Electrophoresis was performed using a 5% Urea-PAGE gel to check the integrity of RNA samples. The purity of RNA samples was determined by measuring the OD260/280 ratios of RNA samples.

RT-qPCR

RNA samples with satisfactory quality were reverse transcribed into cDNA samples using SS-IV-RT system (Invitrogen). To determine the expression of DLGAP1-AS2, qPCRs were performed using SYBR Green Master Mix (Bio-Rad) with 18S rRNA as an internal control.

The expression of mature miR-16 and miR-16 precursor was determined using All-in-One™ miRNA qRT-PCR Reagent Kit (Genecopoeia) with U6 as an internal control. Sequence-specific primers were used in RT-qPCR to determine the expression of miR-16 precursor. To determine the expression of mature miR-16, poly (A) was added to mature miRNAs, followed by using poly (T) as reverse primer to perform reverse transcriptions (RTs), and using poly (T) and sequence-specific forward primer to perform qPCRs.

Each experiment was performed in three technical replicates. The method of $2^{-\Delta\Delta CT}$ was used to normalize Ct values of target genes to corresponding internal controls.

Transwell assay

UWB1.289 cells with transfections were subjected to Transwell assays to determine cell invasion and migration abilities. Corning inserts (8 μm) were used. Briefly, 5000 cells in 100 μl serum-free medium were added into the upper chamber. To induce cell movement, medium supplemented with 20% FBS was added into the lower chamber. To mimic in vivo invasion, membranes were coated with Corning Matrigel for 12 h at 37 °C before invasion assay. Three biological replicates were set for each experiment. At 37 °C, cells were cultivated for 12 h, followed by the collection of membranes. The upper surface was cleaned using cotton swabs, and the lower surface was stained with 0.5% crystal violet (Sigma-Aldrich) for 20 min in dark. Under a light microscope, cells were counted and imaged were taken.

Statistical analysis

Levels of gene expression in OC and paired non-tumor tissues were expressed as average values of three technical replicates, and data were compared by paired t test. Mean +/- SD values of three biological replicates were used to express data of multiple cell transfection groups, and data comparisons were performed by ANOVA Tukey's test. Correlations were analyzed by Pearson's correlation coefficient. $p < 0.05$ was deemed statistically significant.

Results

Altered expression of DLGAP1-AS2, mature miR-16 and miR-16 precursor was observed in OC tissues

OC tissues and paired non-tumor tissues were collected from 62 OC patients included in this study. RT-qPCRs were performed to determine the expression of DLGAP1-AS2, mature miR-16 and miR-16 precursor in these tissue samples. It was observed that DLGAP1-AS2 was significantly overexpressed in OC tissues compared to non-tumor tissues (Fig. 1A, $p < 0.001$). In contrast, mature miR-16 (Fig. 1B, $p < 0.001$) and miR-16 precursor (Fig. 1C, $p < 0.001$) were significantly under-expressed in OC tissues in comparison to non-tumor tissues. Therefore, altered expression of DLGAP1-AS2 and miR-16 may play a role in OC.

DLGAP1-AS2 was inversely correlated with mature miR-16 across OC tissue samples

Correlations between DLGAP1-AS2 and mature miR-16 or miR-16 precursor were analyzed by Pearson's correlation co-efficient. It was observed that, across OC tissues, DLGAP1-AS2 was significantly and inversely correlated with mature miR-16 (Fig. 2A). In contrast, no significant correlation between DLGAP1-AS2 and miR-16 precursor was observed across non-tumor tissues (Fig. 2B). Therefore, altered expression of DLGAP1-AS2 may be related to the maturation of miR-16 from miR-16 precursor to mature miR-16.

DLGAP1-AS2 overexpression suppressed the maturation of miR-16 in UWB1.289 cells

To explore whether DLGAP1-AS2 can affect the maturation of miR-16 UWB1.289 cells were transfected with either DLGAP1-AS2 expression vector or miR-16 mimic, followed by the confirmation of transfections by RT-qPCR (Fig. 3A, $p < 0.05$). It was observed that DLGAP1-AS2 overexpression decreased the expression level of mature miR-16 (Fig. 3B, $p < 0.05$), but showed no significant effects on the expression of miR-16 precursor (Fig. 3C). Moreover, overexpression of miR-16 failed to affect the expression of DLGAP1-AS2 (Fig. 3D). Therefore, DLGAP1-AS2 may suppress the maturation of miR-16 in UWB1.289 cells.

DLGAP1-AS2 overexpression reduced the inhibitory effects of miR-16 overexpression on cell invasion and migration

The role of DLGAP1-AS2 and miR-16 in regulating the invasion and migration of UWB1.289 cells were analyzed by Transwell assay. It was observed that DLGAP1-AS2 overexpression increased the invasion and migration of UWB1.289 cells, while miR-16 overexpression decreased cell invasion (Fig. 4A, $p < 0.05$) and migration (Fig. 4B, $p < 0.05$). Moreover, DLGAP1-AS2 overexpression reduced the inhibitory effects of miR-16 overexpression on cell invasion and migration.

Discussion

In this study we analyzed the involvement of DLGAP1-AS2 in OC, and explored the in vitro interaction between DLGAP1-AS2 and miR-16. We found that DLGAP1-AS2 was significantly overexpressed in OC and it may suppress the maturation of miR-16 to promote cell invasion and migration.

In a recent study Miao et al reported that DLGAP1-AS2 was significantly overexpressed in glioma and its downregulation suppressed the metastasis and growth of tumors but induced cancer cell apoptosis [13]. In addition, the authors found that the functions of DLGAP1-AS2 in glioma are likely mediated by the upregulation of YAP1 [13]. Based on our knowledge, the expression pattern and function of DLGAP1-AS2 in other cancers is unknown. In this study we found that DLGAP1-AS2 was significantly overexpressed in OC, and overexpression of DLGAP1-AS2 increased the invasion and migration of OC cells. Therefore, DLGAP1-AS2 may play oncogenic roles in OC by promoting tumor metastasis.

MiR-16 has been characterized as a tumor suppressor miRNA in different types of cancers, including OC [13, 15]. In OC, miR-16 is downregulated in OC and its overexpression suppresses Wnt/β-catenin signaling pathway to decrease the invasion and migration of cancer cells [15]. Consistently, our study confirmed the downregulation of both mature miR-16 and miR-16 precursor in OC, and the inhibitory effects of miR-16 overexpression on OC cell invasion and migration.

Previous studies of miR-16 mainly focused on the downstream targets of miR-16 in cancer biology [13, 15]. However, the upstream regulators of miR-16, such as the factors regulating the biogenesis, such as the maturation, of miR-16 have not been well studied. In this study we showed that overexpression of DLGAP1-AS2 could suppressed the maturation of miR-16 in OC cells. Although the in vivo interaction between DLGAP1-AS2 and miR-16 is unknown, we observed the inverse correlation between DLGAP1-AS2 and mature miR-16, but not between DLGAP1-AS2 and miR-16 precursor across OC tissues. Therefore, DLGAP1-AS2 may also suppress the maturation of miR-16 in OC patients. However, the mechanism remains elusive. In a recent study Yu et al. showed that lncRNA CCAT2 may suppress the movement of miR-145 precursor from nucleus to cytoplasm, thereby suppressing the maturation of miR-145. Therefore, we speculate that DLGAP1-AS2 may also suppress the transportation of miR-16 in OC cells. However, studies are needed to test our speculation.

In conclusion, DLGAP1-AS2 is overexpressed in OC and it may suppress the maturation of miR-16 to promote OC cell invasion and migration.

Declarations

Ethics approval and consent to participate

The First Affiliated Hospital, School of Clinical Medicine of Guangdong Pharmaceutical University Ethics Committee approved this study. Written informed consent was provided by all patients.

Consent for publication

Not applicable

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Not applicable

Authors' contributions

Ying Tao and Mandan Zhao designed the study. Jie Luo carried out experiments and wrote the manuscipt, Ying Tao and Mandan Zhao revised the paper,Yuqiang Zhang, Ting Zheng, Yongping Jing, Rongyu Cao, Minmin Wu and Die Fan collected patient specimens and related information. Jie Luo, Yuqiang Zhang, Ting Zheng, Yongping Jing, Rongyu Cao, Minmin Wu, Die Fan contributed to analysing the data. All authors reviewed the results and approved the final version of the manuscript.

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Figures

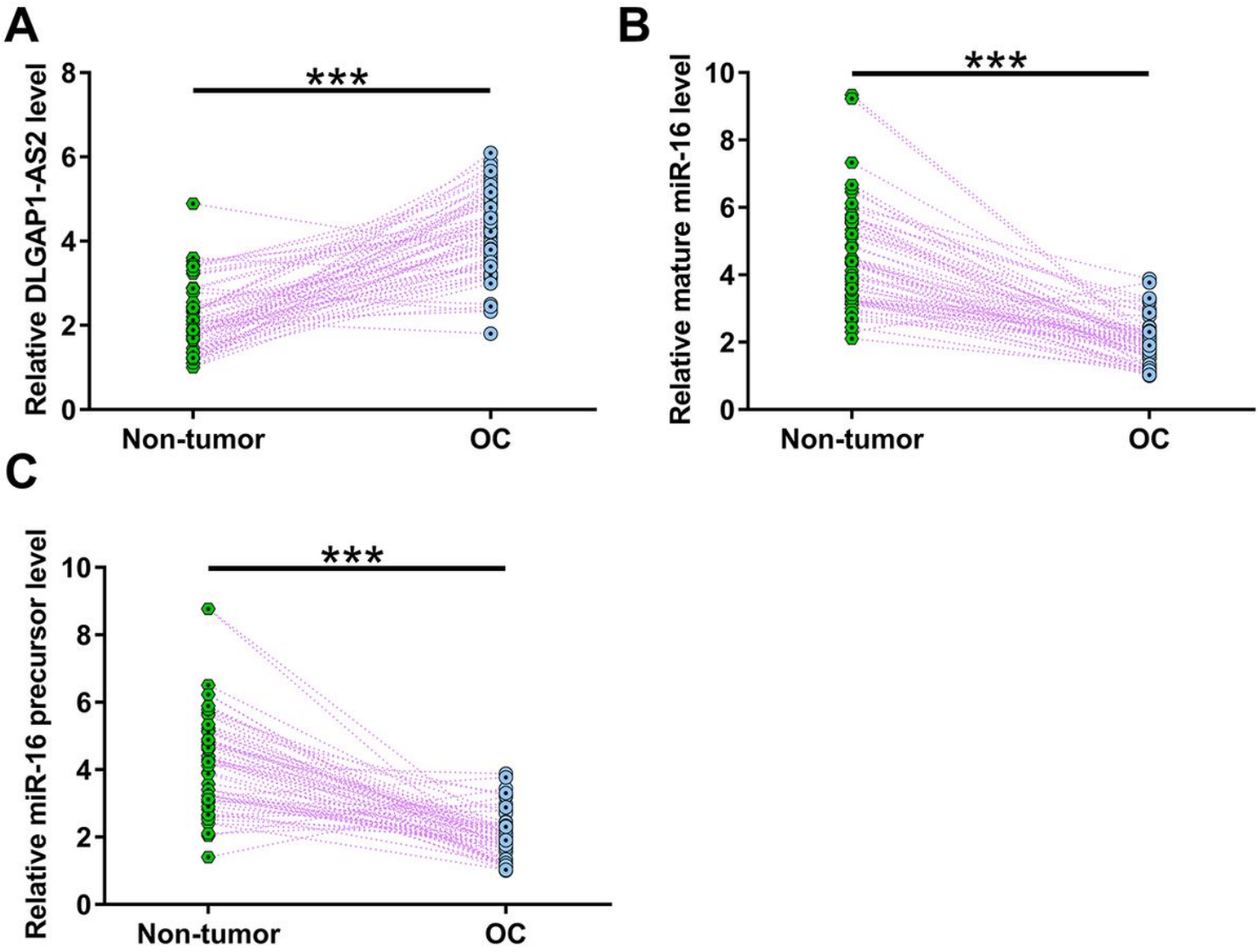

Figure 1

Altered expression of DLGAP1-AS2, mature miR-16 and miR-16 precursor was observed in OC tissues OC tissues and paired non-tumor tissues were collected from 62 OC patients included in this study. RT-qPCRs were performed to determine the expression of DLGAP1-AS2 (A), mature miR-16 (B) and miR-16 precursor (C) in these tissue samples. Levels of gene expression in OC and paired non-tumor tissues were expressed as average values of three technical replicates, ***,p<0.001.

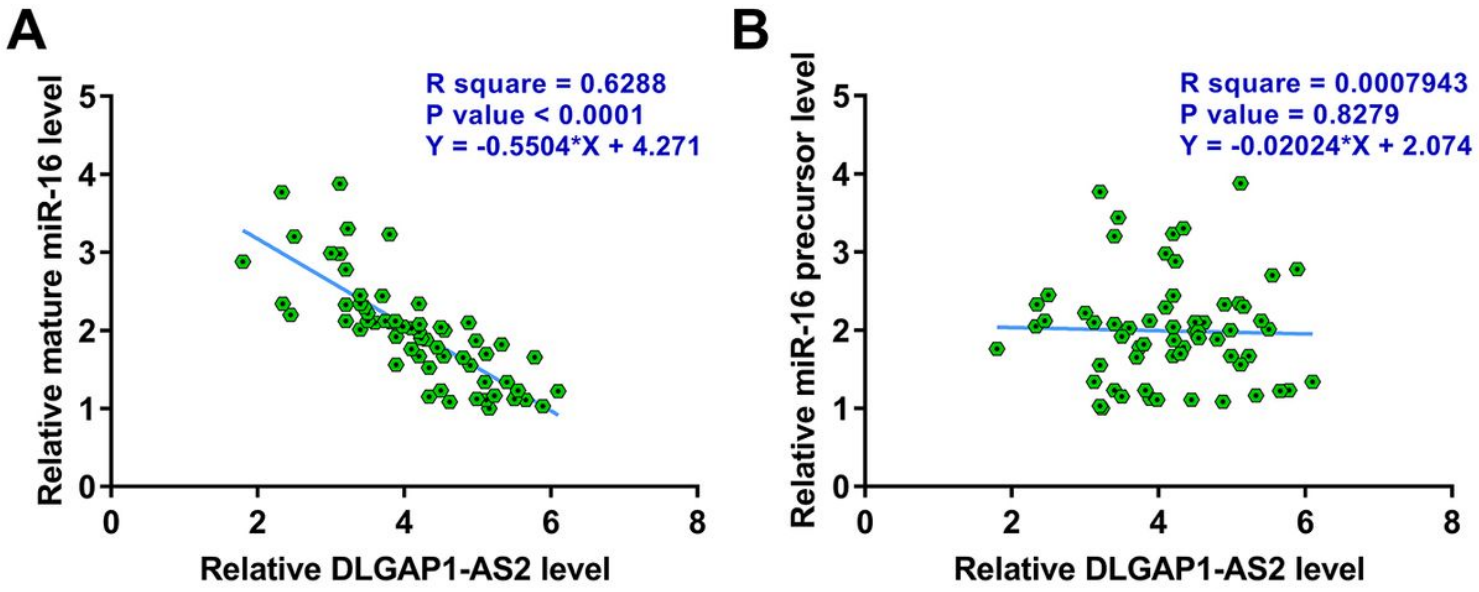

Figure 2

DLGAP1-AS2 was inversely correlated with mature miR-16 across OC tissue samples Correlations between DLGAP1-AS2 and mature miR-16 (A) or miR-16 precursor (B) were analyzed by Pearson's correlation co-efficient.

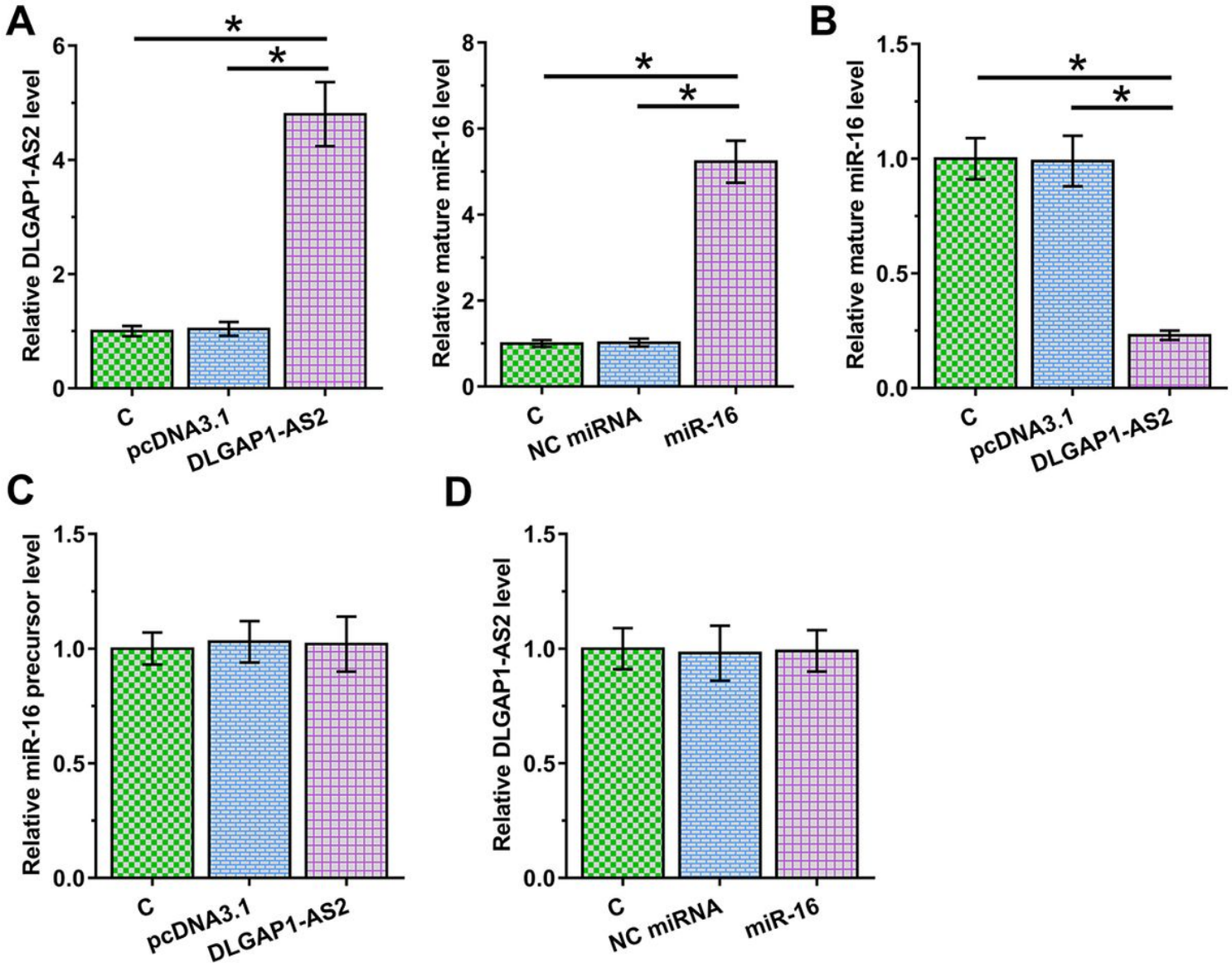

Figure 3

DLGAP1-AS2 overexpression suppressed the maturation of miR-16 in UWB1.289 cells To explore whether DLGAP1-AS2 can affect the maturation of miR-16 UWB1.289 cells were transfected with either DLGAP1-AS2 expression vector or miR-16 mimic, followed by the confirmation of transfections by RT-qPCR (A).The effects of DLGAP1-AS2 overexpression on the expression of mature miR-16 (B) and miR-16 precursor (C), and the effects of miR-16 overexpression on the expression of DLGAP1-AS2 (D) were also analyzed by RT-qPCR. Mean +/- SD values of three biological replicates were used to express data of multiple cell transfection groups.*,$p<0.05$.

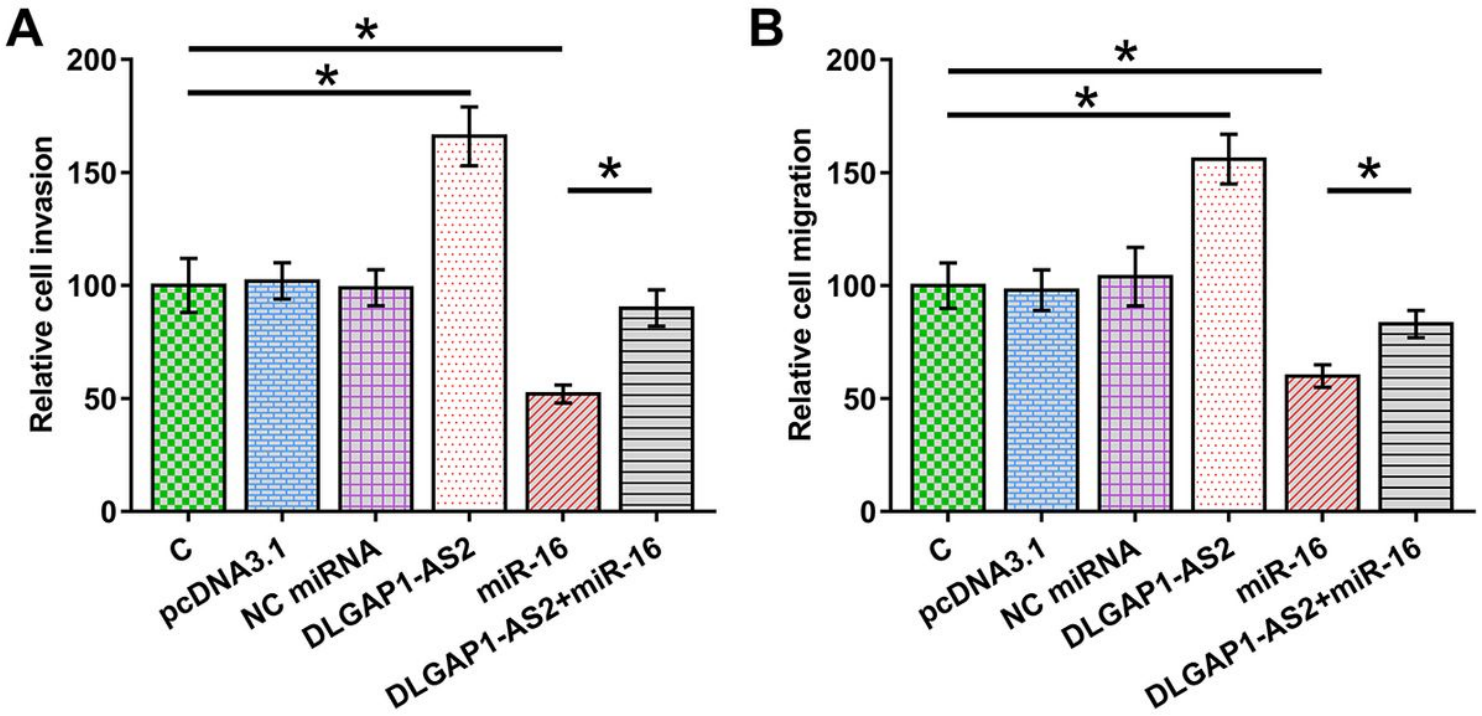

Figure 4

DLGAP1-AS2 overexpression reduced the inhibitory effects of miR-16 overexpression on cell invasion and migration The role of DLGAP1-AS2 and miR-16 in regulating the invasion (A) and migration (B) of UWB1.289 cells were analyzed by Transwell assay. Mean +/- SD values of three biological replicates were used to express data of multiple cell transfection groups.*,$p<0.05$.