Multidrug resistance severely limits the efficacy of ovarian cancer (OC) treatment. Recent studies have revealed the carcinogenic role of LINC00707 RNA. However, the role of LINC00707 in the development of multidrug resistance in OC has not been clarified. Therefore, the aim of this study was to investigate the relationship between LINC00707 and multidrug resistance in OC, which can facilitate the development of new therapeutic agents for effectively addressing this issue. The RNA expression of LINC00707, miR-382-5p and leucine-rich repeat kinase 2 (LRRK2) in SKOV3 (a human OC cell line) cells was detected by qRTPCR. The effects of LINC00707 on the proliferation and viability of SKOV3 cells were determined by MTT assay and colony formation assay. The interaction of LINC00707, miR-382-5p, and LRRK2 was bioinformatically predicted and verified with dual-luciferase reporter assay. In addition, the effect of LINC00707 on drug resistance in SKOV3 cells through targeting the miR-382-5p/LRRK2 axis was explored. The expression of LINC00707 and LRRK2 was significantly increased in SKOV3 cells, while miR-382-5p expression was significantly decreased. The results of bioinformatic prediction and colony formation assay demonstrated that LINC00707 could regulate LRRK2 expression in SKOV3 cells by targeting miR-382-5p. Additionally, knockdown of LINC00707 markedly increased expression of miR-382-5p and decreased that of LRRK2, increased cell proliferation and viability, as well as sensitivity to chemotherapeutic agents in SKOV3 cells. Notably, these manifestations were more obvious with simultaneous knockdown of LINC00707 and miR-382-5p compared with knockdown of LINC00707 alone. LINC00707 is overexpressed in SKOV3 cells and promotes SKOV3 cell proliferation and resistance to chemotherapeutic drugs via targeting the miR-382-5p/LRRK2 axis.

Keywords: ovarian cancer; LINC00707; miR-382-5p; LRRK2; multidrug resistance

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also increases the susceptibility of cancer cells to medication resistance.

MicroRNAs (miRNAs), small non-coding RNAs of about 20 nucleotides in size, regulate the expression level of genes by binding to the 3' UTR region of target genes, thereby affecting the regular activities of cells (Sun et al., 2019). MiRNAs may play a direct or indirect role in the progression of cancer and the emergence of drug resistance to chemotherapeutics. A study showed that downregulated miR-130a was associated with multidrug resistance in various cancers, and miR-130a targeted MET and enhanced TRAIL sensitivity in NSCLC cells (Acunzo et al., 2012). According to the results of previous studies, miR-382-5p may be a tumor suppressor gene, and reduction of its expression can promote cancer progression (Xie & Pan, 2021). Furthermore, increased miR-382-5p expression levels led to significant reduction in iron levels in OC cells (Sun et al., 2021). However, no studies have yet been conducted on the role of miR-382-5p in acquisition of multidrug resistance in OC cells. Interestingly, an interaction between LINC00707 and miR-382-5p has been revealed, which is shown to affect cancer progression (Guo et al., 2021), but the role of this relationship in OC remains unclear. We proposed the multidrug resistance mechanism of OC as follows: LINC00707 acted as a molecular sponge for miR-382-5p and increased OC progression and resistance to chemotherapeutic agents by targeting miR-382-5p. This hypothesis was further supported by in vitro cell experiments.

MATERIALS AND METHODS

Cell culture and treatment

Human normal ovarian surface epithelial cell line (IOSE80) and human OC cell line (SKOV3) were obtained from the American Type Culture Collection (ATCC, VA, USA). The cells were cultured in DMEM/F12 medium supplemented with 100 U/mL penicillin, 0.1 mg/mL streptomycin sulfate, and 10% fetal bovine serum (FBS), then the medium was incubated at 37°C and 5% CO₂.

According to the manufacturer’s instructions, the following plasmids or fragments were transfected into the cells using Lipofectamine™ 2000 transfection reagent: control shRNA, LINC00707 shRNA, control pcDNA3.1 vector, pcDNA3.1-LINC00707, control mimics, miR-382-5p mimics, control inhibitor, and miR-382-5p inhibitor. Next, LINC00707 shRNA and miR-382-5p inhibitor were transfected into SKOV3 cells together, upon which the following groups were obtained: sh-NC, sh-LINC00707, control vector, LINC00707, miR-NC, miR-382-5p, Anti-NC, Anti-miR-382-5p, and sh-LINC00707 + Anti-miR-382-5p. The medium was replaced with fresh medium six hours after transfection for two days of culture. Finally, the transfection efficiency was verified using qRT-PCR.

Quantitative real-time PCR (qRT-PCR)

The TRIzol™ Plus RNA Purification Kit was used to extract total RNA from SKOV3 cells following the manufacturer’s instructions. The concentration and purity of RNA were subsequently measured by NanoDrop One ultramicrospectrophotometer. With the help of PrimeScript RT Master Mix, cDNA was obtained by reverse transcription. qRT-PCR was performed to detect the expression levels of LINC00707, miR-382-5p, and leucine-rich repeat kinase 2 (LRRK2) according to the SYBR Premix Ex Taq II kit. GAPDH or U6 served as an internal control, and six replicates were set up for the experiment. The experimental data obtained by qRT-PCR were used to calculate the relative expression of the target gene with the help of the 2^−ΔΔCt method. The primer sequences used are shown in Table 1.

MTT assay

The transfected SKOV3 cells were seeded in a 96-well plate at a density of 5×10^4 cells/well, and to each well, 20 μL MTT solution (5 mg/mL) was added. After 4 h of incubation at 37°C, the supernatant was aspirated and 200 μL DMSO was then added into each well. Later, the absorbance values at a wavelength of 490 nm were measured by a microplate reader, and the cell viability was evaluated. In order to observe the drug resistance of cells, the transfected SKOV3 cells were incubated with different concentrations of paclitaxel (PTX, 2, 4, 8, 16, 32 μmol/L), doxorubicin (Dox, 0.5, 1, 2, 4, 8 μmol/L), methotrexate (MTX, 2, 4, 8, 16, 32 μmol/L), and cisplatin (DDP, 0.25, 0.5, 1, 2, 4 μmol/L) for 48 h. Subsequently, the addition of 20 μL MTT solution to each well, the supernatant was aspirated after 4 h of incubation at 37°C. Again, 200 μL DMSO was added to each well, and the absorbance at 590 nm was detected by a microplate reader. Half-maximal inhibitory concentration (IC50) was calculated with the use of GraphPad Prism 7 software.

Colony formation assay

The transfected SKOV3 cells were inoculated into 6-well culture plates at 1×10⁴ cells/well and cultured in DMEM/F12 medium containing 10% FBS for two weeks, with the medium replaced every three days. Afterwards, SKOV3 cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet.

Dual-luciferase reporter assay

In order to verify the interaction among LINC00707, miR-382-5p, and LRRK2, wild type LINC00707 and LRRK2, or LINC00707 and LRRK2 fragments with

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<th>Table 1. Primer Sequences for qRT-PCR</th>
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**LINC00707 promotes multidrug resistance of ovarian cancer cells**

Mutated nucleic acid sequences were constructed and then inserted into the luciferase reporter gene of pMIR-REPORT (pGL3) plasmid (H306, Obio Technology, Shanghai, China) based on the predicted binding sites of LINC00707 and LRRK2 with miR-382-5p. The cells were accordingly divided into four groups: wild type pGL3-LINC00707 (LINC00707-WT) and pGL3-LRRK2 (LRRK2-WT) or mutant pGL3-LINC00707 (LINC00707-MUT) and pGL3-LRRK2 (LRRK2-MUT) vectors. Next, miR-382-5p mimics and pGL3 plasmids were transfected together into SKOV3 cells using Lipofectamine™ 2000. Subsequently, luciferase activity was assessed using the Dual-Luciferase Reporter System Kit (E1910, Promega, USA).

**Western blot**

The cells were lysed in lysis buffer (RIPA) supplemented with a protease inhibitor (PMSF). Equal amounts of protein (20 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. After that, the membranes were blocked with 5% milk in Tris-buffered saline containing 0.1% Tween-20 for 1 h at ambient temperature. The membranes were subsequently incubated overnight at 4°C with primary LRRK2 antibody (1:1000, ab133474, Abcam, Cambridge, UK), followed by 1 h of incubation at 37°C with peroxidase conjugated goat anti-rabbit immunoglobulin G (IgG) antibody (1:3000; ab97051). Next, immunoreactive bands were visualized using enhanced chemiluminescence reagents (Thermo Fisher Scientific, Waltham, USA) and imaged using a Luminescent Image Analyzer ChemiDoc XRS Plus (Bio-Rad). Optical densitometric quantification of band intensities from four independent experiments was performed using Image-Pro Plus 6.0 software, and relative expression levels of target proteins were normalized to band intensities of GAPDH.

**Data analysis**

Measurement data were presented as mean ± standard deviation (S.D.). The t-test was adopted when comparing only two groups, and one-way analysis of variance with Tukey’ test was employed when comparing more than two groups. Pearson correlation was applied to analyze the expression correlation. All statistical analyses were performed using SPSS 26.0 software (IBM, Armonk, NY, USA), with two-tailed p<0.05 as the level of statistical significance. All experiments were performed in triplicate, with at least three independent experiments.

**RESULTS**

**LINC00707 is upregulated in SKOV3 cells**

QRT-PCR was used to determine LINC00707 expression in OC cells in this study. We found that LINC00707 was expressed at significantly higher levels in SKOV3 than in IOSE80 cells (Fig. 1), which suggests that LINC00707 may be involved in OC development.

**Knockdown of LINC00707 suppresses the proliferation of SKOV3 cells**

In order to investigate the effect of LINC00707 on OC, LINC00707 was knocked down or overexpressed using plasmids, and the transfection efficiency was detected by qRT-PCR. The qRT-PCR results demonstrated that the expression level of LINC00707 was significantly decreased in cells in the sh-LINC00707 group compared with that in the sh-NC group and was notably increased.
Figure 3. Knockdown of LINC00707 decreases multidrug resistance in ovarian cancer (OC) cells. 
(A–D) MTT assay results of the effect of PTX (A), Dox (B), MTX (C) and DDP (D) on drug resistance in SKOV3 cells with knocked down or overexpressed LINC00707. **p<0.01, vs sh-NC; iip<0.01, vs Vector. sh, short hairpin; NC, negative control; PTX, paclitaxel; Dox, doxorubicin; MTX, methotrexate; DDP, cisplatin.

Figure 4. LINC00707 targets miR-382-5p and negatively interacts with miR-382-5p in ovarian cancer (OC) cells. 
(A) The binding sites between LINC00707 and miR-382-5p predicted by lncBASE software. 
(B) Dual-luciferase reporter assay results of relative fluorescence activity in SKOV3 cells transfected with LINC00707 WT + miR-382-5p mimics and LINC00707 MUT + miR-382-5p mimics. 
(C) qRT-PCR results of the expression level of miR-382-5p in SKOV3 cells transfected with LINC00707. 
(D) qRT-PCR results of the expression levels of miR-382-5p in IOSE80 cells and SKOV3 cells. **p<0.01, vs. sh-NC (or miR-382-5p NC); iP<0.01, vs Vector. WT, wild type; MUT, mutant; sh, short hairpin; NC, negative control.
LINC00707 promotes multidrug resistance of ovarian cancer cells

in the LINC00707-oe group compared with the control vector group (Fig. 2A). Later, we examined cell proliferation and viability through MTT and colony formation assays. Combining the results of both assays, we observed that the proliferation and viability of cells in the sh-LINC00707 group were much lower than those in the sh-NC group, and the proliferation and viability of cells in the LINC00707-oe group were much higher than those in the control vector group (Fig. 2B, 2C). Thus, knockdown of LINC00707 can significantly inhibit SKOV3 cell proliferation.

Knockdown of LINC00707 decreases multidrug resistance in SKOV3 cells

Subsequently, in order to observe the effect of LINC00707 on chemoresistance of SKOV3 cells, the resistance of LINC00707-knock-down and LINC00707 overexpressing SKOV3 cells to PTX, Dox, MTX, and DDP was studied using MTT assay. The MTT results showed that the IC50 of SKOV3 cells treated with PTX, Dox, MTX, and DDP was markedly decreased in the sh-LINC00707 group compared with the sh-NC group, while being considerably increased in the LINC00707-oe group relative to the control vector group (Fig. 3A–D). In brief, knockdown of LINC00707 can reduce the resistance of SKOV3 cells to chemotherapeutic agents.

LINC00707 targets miR-382-5p

LINC00707 was predicted to target miR-382-5p using lncBASE software (Fig. 4A). According to the findings of dual-luciferase reporter assay, co-transfection of WT-LINC00707 3’-UTR with miR-382-5p mimics in the miR-382-5p group greatly reduced the luciferase activity compared with the miR-NC group (Fig. 4B). qRT-PCR results indicated that SKOV3 cells in which LINC00707 was knocked down showed significant increase in the miR-382-5p expression compared with the cells in the sh-NC group, and those with LINC00707 overexpression showed significant downregulation of miR-382-5p expression compared with those in the control vector group (Fig. 4C). In addition, the expression level of miR-382-5p was much lower in SKOV3 cells than in IOSE80 cells (Fig. 4D). Overall, LINC00707 targets miR-382-5p and sh-LINC00707 can significantly upregulate the miR-382-5p expression in OC.

LRRK2 is a target gene of miR-382-5p

Firstly, we generated SKOV3 cells with miR-382-5p knock down or overexpression to determine the relationship between miR-382-5p and LRRK2. The expression level of miR-382-5p in SKOV3 cells was significantly elevated in the miR-382-5p group compared with the miR-NC group and the expression level of miR-382-5p was markedly lowered in the Anti-miR-382-5p group relative to the Anti-NC group (Fig. 5A). Subsequently, with the help of starbase2.0 (https://starbase.sysu.edu.cn/starbase2/index.php), miR-382-5p was predicted to target LRRK2 mRNA (Fig. 5B). The dual-luciferase reporter assay results showed that the miR-382-5p group presented a significant decrease in luciferase activity after co-transfection of the WT-LRRK2 3’-UTR with miR-382-5p mimics (Fig. 5C). qRT-PCR results indicated that miR-382-5p overexpression could considerably reduce LRRK2 expression compared with the miR-NC group in SKOV3 cells, while miR-382-5p knockdown significantly upregulated LRRK2 expression compared with the Anti-
NC group (Fig. 5D). Additionally, LRRK2 was expressed at quite higher levels in SKOV3 cells than in IOSE80 cells (Fig. 5E). Collectively, we found that miR-382-5p expression and LRRK2 expression are upregulated in OC, miR-382-5p directly targets LRRK2, and knockdown of miR-382-5p significantly promotes LRRK2 expression.

**LINC00707 promotes proliferation and multidrug resistance of SKOV3 cells by targeting the miR-382-5p/LRRK2 axis**

In order to further investigate the effect of LINC00707 on OC cell viability and drug resistance, the expression level of LRRK2 was detected by qRT-PCR and Western blot by targeting the miR-382-5p/LRRK2 axis, after simultaneous knockdown of LINC00707 and miR-382-5p in SKOV3 cells. The results showed that the sh-LINC00707 + Anti-miR-382-5p group displayed a much higher expression level of LRRK2 than the sh-LINC00707 group, and the expression level of LRRK2 was significantly decreased in the sh-LINC00707 + Anti-miR-382-5p group compared with the Anti-miR-382-5p group (Fig. 6A, 6B). The MTT assay outcomes indicated that cell viability was considerably increased in the sh-LINC00707 + Anti-miR-382-5p group compared with the sh-LINC00707 group, but significantly reduced in the sh-LINC00707 + Anti-miR-382-5p group compared with the Anti-miR-382-5p group (Fig. 5C). Next, the resistance of SKOV3 cells to chemotherapeutic agents (PTX, Dox, MTX, and DDP) was detected using MTT assay. We discovered that the sh-LINC00707 + Anti-miR-382-5p group had a much higher IC50 than the sh-LINC00707 group, and the sh-LINC00707 + Anti-miR-382-5p group presented a lower IC50 than the Anti-miR-382-5p group (Fig. 6D–G). These results suggest that LINC00707 promotes proliferation and resistance of SKOV3 cells to chemotherapeutic agents by targeting the miR-382-5p/LRRK2 axis.

**DISCUSSION**

OC, one of the gynecological malignancies with the highest mortality rates, poses a severe threat to female health worldwide. Research suggests that chemoresistance of OC cells significantly contributes to the high mortality (Barriga-Rivera et al., 2016). Currently, the combination of Dox and PTX is typically effective at first, but the
condition relapses in the majority of patients, and is then usually incurable, with just an 18-month-survival rate (Yang et al., 2017). Therefore, a thorough understanding of the molecular mechanism of multidrug resistance in OC is crucial for improving the prognosis. Our study’s findings showed that LINC00707, which was expressed at a considerably higher level in OC, could boost cellular resistance to chemotherapeutic drugs by targeting the miR-382-5p/LRRK2 axis.

LncRNAs, described in recent studies, hold promise as potential biomarkers for cancer diagnosis, prognosis, and treatment. Notably, many lncRNAs are dysregulated in OC, which may play crucial roles in the incidence and progression of tumors (Wang et al., 2018). Among them, lncRNA HOXD-AS1 is an OC-associate lncRNA that is overexpressed in both OC tissues and cells and can indicate poor prognosis in patients (Zhang et al., 2017). Recently, Zahra and coworkers performed high-throughput sequencing and found that LINC00707 was highly upregulated in OC tissues and has potential as a diagnostic marker (Zahra et al., 2021). Although earlier research indicated that LINC00707 is a proto-oncogene (Guo et al., 2021), it is not clear how LINC00707 and OC are related. Therefore, the aim of our study was to investigate the effect of LINC00707 in OC and its molecular mechanism. In this study, LINC00707 was overexpressed in SKOV3 cells, and LINC00707 overexpression markedly increased the proliferation and viability of SKOV3 cells, while LINC00707 knockdown inhibited the proliferation and viability of cells. Furthermore, studies on several malignancies have shown that lncRNAs have a role in controlling chemosensitivity (Xu et al., 2018). Our findings clearly showed that LINC00707 overexpression increased resistance to PTX, Dox, MTX, and DDp in SKOV3 cells. These findings are in line with the results of LINC00707 overexpression in cancer cells in the bladder (Gao & Ji, 2021) and breast (Yuan et al., 2020). Thus, it is clear that LINC00707 overexpression induces OC resistance to chemotherapeutic agents.

Recent evidence has suggested that the interaction between lncRNAs and microRNAs may have an impact on a number of pathological mechanisms, including the development of cancer and the acquisition of drug resistance. By functioning as a competitive endogenous RNA sponge for miR-17 and altering STAT3 expression, lncRNA H19 was discovered by Hu et al. to regulate the development of NSCLC (Huang et al., 2018). Li et al. discovered that lncRNA SNHG1 promoted the resistance of hepatocellular carcinoma cells to sorafenib by increasing miR-21 expression to activate the Akt pathway (Li et al., 2019). Through bioinformatic predictions and several experiments in our study, we demonstrated that there was a binding site in LINC00707 for miR-382-5p, and LINC00707 acted as the molecular sponge of miR-382-5p. Interestingly, Guo et al. also proved the interaction between LINC00707 and miR-382-5p (Wang et al., 2020). However, our study is the first to find that miR-382-5p expression is significantly downregulated in OC cells and inversely correlated with LINC00707 expression. Although miR-382-5p has only been the subject of a few studies, they have shown that this microRNA suppresses the genes involved in tumor development. For instance, the expression level of miR-382-5p is significantly downregulated in colorectal cancer tissues (Xie & Pan, 2021). Likewise, few studies were conducted on the downstream target genes of miR-382-5p. In this regard, our study discovered that miR-382-5p targeted the LRRK2mRNA 3’-UTR region and regulated the expression level of LRRK2. Mutations in LRRK2 were first thought to be critical for inducing familial Parkinson’s disease (Deniston et al., 2020). Interestingly, the expression level of LRRK2 was found to be significantly downregulated in NSCLC tissues (Ma et al., 2019). However, the role of LRRK2 in cancer has rarely been reported. In our study, the expression level of LRRK2 was discovered to be significantly upregulated in SKOV3 cells and was clearly inversely regulated by miR-382-5p. LRRK2 expression level in SKOV3 cells was significantly higher when LINC00707 and miR-382-5p were simultaneously knocked down than that when either of them was knocked down alone. Moreover, SKOV3 cells in the sh-LINC00707 + Anti-miR-382-5p group were significantly more resistant to chemotherapeutic agents than those in the Anti-miR-382-5p group, but significantly less resistant than those in the sh-LINC00707 group. Therefore, LINC00707 induced multidrug resistance of SKOV3 cells by targeting the miR-382-5p/LRRK2 axis.

CONCLUSION

In summary, LINC00707 is highly expressed in SKOV3 cells. LINC00707 regulates the expression of LRRK2 by targeting miR-382-5p, thereby enhancing the proliferation and viability of SKOV3 cells and inducing multidrug resistance in them. Therefore, knocking down LINC00707 expression is key for addressing multidrug resistance in OC cells. However, the role of LINC00707 is multifaceted, and more research is required to fully elucidate its function.

Declarations

Conflict of Interest. All authors declare no conflict of interest.

Consent for Publication. Not applicable.

Authors’ Contributions. Min-Wen Zhao conceived and designed the experiments; Chang-Jie Lin analyzed and interpreted the results of the experiments; YZM performed the experiments; and Jian-Ping Qiu revised the manuscript.

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