Article

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CircRNA *Hsa_circ_0120175* Promotes Ovarian Cancer Tumorigenesis and Predicts a Poor Prognosis

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Background: Circular RNA is a type of non-coding RNA that is commonly found in eukaryotic genomes. They play an essential role in the biological processes of cell proliferation and cell apoptosis involved in normal development and abnormal tumorigenesis. In this study, we examined whether that the circular RNA GENE *hsa_circ_0120175* is substantially expressed in epithelial ovarian cancer, and then explored whether *hsa_circ_0120175* plays an important role in the occurrence and development of ovarian cancer.

Methods: A total of 40 patients with ovarian cancer were included in this study, and tissue samples were collected from both ovarian cancer tissues and paracancer tissues. The levels of *hsa_circ_0120175* expression were determined using qRT-PCR in both varian cancer cells and tissues. This study assessed the impact of *hsa_circ_0120175* on cellular proliferation, migration, and invasion using various *in vitro* assays with cultured ovarian carcinoma cells.

Results: *Hsa_circ_0120175* was highly expressed in both human tissues and ovarian cancer cells. In ovarian cancer patients, the expression level of *hsa_circ_0120175* was significantly different among The International Federation of Gynecology and Obstetrics (FIGO) stages ($p = 0.03$), and the difference was statistically significant. Multivariate Cox regression analysis showed that lymph node metastasis was independently related to Overall Survival (OS), and the difference was statistically significant ($p = 0.033$). *In vitro*, decreasing *hsa_circ_0120175* significantly reduced ovarian carcinoma cell proliferation, migration, and invasion ($p < 0.05$).

Conclusions: *Hsa_circ_0120175* has the potential to serve as a biomarker for diagnosing and treating ovarian carcinoma. This is because it promotes cellular proliferation, migration, and invasion in epithelial ovarian carcinoma.

Keywords: circular RNA (circRNA); oncogene; ovarian cancer (OC); invasion

Introduction

Ovarian cancer (OC) is the deadliest malignant tumor of the female reproductive system [1]. It is the sixth most prevalent cancer in women worldwide as of 2018 [2]. OC is usually asymptomatic in the early stage, lacks biomarkers for effective early diagnosis, and progresses insidiously and rapidly. When diagnosed, more than 80% of OC patients are already in the terminal cancer stage (III or IV) [3]. This poor prognosis and high mortality rate seriously threaten women's health and lifespan [4,5]. Therefore, it is imperative to further study the underlying mechanisms of OC initiation and development and to find tumor biomarkers for early diagnosis or potential therapeutic targets.

Circular RNA (circRNA) is a type of non-coding RNA with a closed-loop structure that was first discovered in eukaryotes in 1976 [6]. Circular RNAs (circRNAs) have a high expression level, high stability, and tissue specificity [7,8]. They regulate linear RNA transcription and protein translation, performing a variety of different biological functions [9]. Circular RNAs have been shown to have both oncogenic and tumor-suppressive functions, playing a central regulatory role in tumorigenesis and progression. They have been suggested as biomarkers for tumor diagnosis or new therapeutic targets [10]. For instance, *hsa_circ_0005273* influences the YAP1-Hippo signaling pathway, promoting breast cancer carcinogenesis

[11]. CircCELSR1 promotes paclitaxel resistance in OC cells through the circCELSR1-miR-1252-FOXR2 regulatory axis [12]. Additionally, *hsa_circ_0077837* is substantially related to clinicopathological characteristics and a poor prognosis in bladder cancer patients by interfering with the biological processes of bladder cancer cells [13].

In this study, we used RNA sequencing (RNA-seq) to analyze total RNA from three pairs of OC and surrounding non-cancerous ovarian tissue samples. We characterized the circRNA transcripts and detected that *hsa_circ_0120175* was overexpressed in OC tissues. Subsequently, we confirmed that the expression level of *hsa_circ_0120175* was significantly higher in OC tissues than in the nearby non-cancerous ovarian tissues in a sizable cohort of our samples. Our study suggests that *hsa_circ_0120175* may be a promising biomarker for OC diagnosis and a possible therapeutic target.

Materials and Methods

Patient Samples

This study was conducted from September 2017 to June 2018 at the First Affiliated Hospital of Wannan Medical College (Yijishan Hospital of Wannan Medical College) involving 80 patients. Before surgery, each patient provided an informed consent form and confirmed that they had not undergone preoperative radiotherapy. The study included 40 OC tissues and 40 control tissues derived from the ovarian cancer adjacent tissues. Two independent pathologists at The First Affiliated Hospital of Wannan Medical College (Yijishan Hospital of Wannan Medical College) confirmed the diagnosis of all samples. The Ethics Committee of the Yijishan Hospital approved the study (the name of the ethics committee: 2022 Lun Review Research No. 04).

The inclusion criteria were the following: the patient did not receive any radiotherapy or chemotherapy before surgery, anesthesiology and imaging evaluation before surgery could be performed, and the ovarian malignant tumor was confirmed by histopathology after surgery.

The exclusion criteria were the following: patients who had received preoperative radiotherapy or chemotherapy, had other tumors, serious medical and surgical diseases, and were pregnant or lactating women.

RNA-seq and Identification of circRNAs

RNA-seq and identification of circRNAs were performed using established methods [14]. Total RNA was extracted from tissues or cells using RNAiso Plus (Cat. no: 9109, Takara, Shiga, Japan). The quality and quantity of the samples were verified. Libraries were created using the KAPA RNA HyperPrep Kit with RiboErase (HMR) (Illumina, New England Biolabs, Beverly, MA, USA). The libraries were then sequenced using NovaSeq6000 (Illumina Novaseq6000, Illumina, San Diego, CA, USA). Finally, effective reads were mapped against the human reference genome (GRCh37/hg19) for circRNA identification using bioinformatic methods.

Cell Culture

One normal ovarian cell line, IOSE-80 cell, and two human OC cell lines, SKOV3 and HO8910, were obtained from the Shanghai Institute of Drug Research (Shanghai, China). The short tandem repeats (STR) results of all cell lines were consistent, and no mycoplasma infection was found. HO8910 and IOSE-80 cell lines were cultured in RPMI1640 culture medium (Cat. no: 11875093, Gibco, Beijing, China), while SKOV3 cells were cultured in McCoy's 5A culture medium (Cat. no: 16600082, Gibco, Beijing, China). Both media were supplemented with 10% fetal bovine serum (FBS) (Cat. no: F8318, Sigma, St. Louis, MO, USA) and 1% penicillin-streptomycin antibiotics. Cells were incubated and frequently observed using an inverted light microscope (Cat. no: DM6 FS, Axio Imager: Carl Zeiss, Oberkochen, Germany). The cells were passaged by EDTA/trypsin (Cat. no: 252000-056, Gibco, Grand Island, NY, USA) digestion when their confluency reached 80%.

Cell Transfection

GENESEED (Guangzhou, China) provided two types of small interfering RNA (siRNA)—one targeting *hsa_circ_0120175* and the other being a negative control siRNA (si-NC). SKOV3 and HO8910 cells were seeded into 6-well plates at a density of 1.5×10^5cells and incubated at 37 °C (98.6 °F) until they reached a cell density of about 50%. The cells were then transfected with the siRNAs using Lipofectamine™ 3000 Transfection Reagent (Cat. no: L3000015, Invitrogen, Waltham, MA, USA) following the manufacturer's instructions. The transfection sequence used was 5′-AGTTTGCGGACAATGTGTC-3′, 3′-TCAAACGCCTGTTACACAG-5′.

RNA Extraction and RT-qPCR

TRIzol (Invitrogen, Waltham, MA, USA) was used to harvest total RNA from ovarian cells and tissues. RNAiso Plus (Cat. no: 9010, Takara, Kusatsu, Japan), and NanoDrop 2000 were used to assess the samples' purity and concentration (Cat. no: NanoDrop 2000, Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription of total RNA to first-strand cDNA using the First Strand cDNA Synthesis Kit (GS0201-2, Geneseed, Guangzhou, China) was conducted. Finally, the first-strand cDNA samples were mixed with a SYBR Green qPCR Mix (GS0201-3, Geneseed, Guangzhou, China) and subjected to qRT-PCR on an ABI 7900HT sequencer (Cat. no: 7900HT, Thermo Fisher Scientific, Waltham, MA, US). The experimental results were calculated using the $2^{-\Delta\Delta Ct}$ method. At least three independent replications of the experiment were accomplished. The following primers

were used: the forward primer (*hsa_circ_0120175*-F1): 5′-ATGATAGTAAAAGTTTGCGGACAAT-3′ and the reverse primer (*hsa_circ_0120175*-R1): 5′-GTGCACCAACTGAAGTACAC-3′.

Nuclear/Cytoplasmic Fractionation

The nuclear and cytoplasmic fractions of cells were isolated using the nuclear/cytoplasmic fractionation kit (Cat. no: AM1921, Life Technologies, Carlsbad, CA, US). The cells were digested using trypsin, washed in phosphate-buffered saline (PBS), and incubated in the cell fractionation buffer of the kit. After centrifugation, the supernatant was the cytoplasmic fraction, while the nuclear fraction was extracted from the pellet using the nuclear extraction buffer of the kit.

Cell Viability Assay

In 96-well plates, 4×10^4 SKOV3 and HO8910 cells were planted, and the plates were then cultured in an incubator for 24 hours. At 1, 2, 3, and 4 days, correspondingly, 10 μL of Cell Counting Kit-8 (CCK-8, WST-8, Tokyo, Japan) was added to each well. Then, the cells were treated with CCK-8 for 2 hours in the incubator, and then the absorbance (OD) at 450 nm was recorded using a spectrophotometric microplate reader (Epoch, BioTek, Winooski, VT, USA).

Colony Formation Assay

Cells were seeded in 6-well plates at a density of 200 cells per well and incubated for 24 h. After transfection, the cells were grown for two weeks at 37 °C (98.6 °F) in an incubator with 5% CO_2. The cell culture medium was changed every three days. The cells were washed with PBS (Cat. no: P1003, Solarbio, Beijing, China), and colonies were fixed using paraformaldehyde (Cat. no: P0099, Beyotime Biotechnology, Shanghai, China) for 30 min and then stained with crystal violet (Cat. no: 1.01408.0025, Millipore, Billerica, MA, USA) for 15 min.

Transwell® Assay

Cells were cultured in a Transwell® chamber (Corning, 24-well insert, 8-micron pore size) with or without Matrigel (Cat. no: 354263, Corning, Ithaca, NY, USA) to examine cellular migration and invasion. Cells were passaged and cultured in a Transwell® chamber at a density of 1×10^5 cells/mL in serum-free medium, and a complete medium was added to the lower layer of each chamber. After 24 h, the cells on the membrane's underside were stained with 0.1% crystal violet (Cat. no: 1.01408.0025, Millipore, Billerica, MA, USA) and tallied in five arbitrary high-power fields of view under a microscope.

Wound Healing Assay

To examine the groups of cells, they were resuspended and inoculated in 6-well plates (5×10^5 cells per well). Once the cells had reached 90–100% confluence, a vertical scratch was made in each well using a 200 µL pipette tip. After rinsing off excess suspended cells with phosphate-buffered saline (PBS), 2 mL of serum-free medium was added to each well. The cells were then cultured at 37 °C (98.6 °F) and incubated in an incubator with 5% CO_2. Images were taken of the scratch wounds at 0, 12, and 24 h, and the wounded area was measured.

Statistical Analysis

The statistical analysis was performed using SPSS (SPSS24.0, IBM, Armonk, NY, USA) and GraphPad Prism (GraphPad Prism 5.0, GraphPad Software, San Dieg, CA, USA) was used for graphing. The group design *t*-test was used for comparisons between two groups, while the chi-square/Fisher analysis was utilized for comparisons among multiple groups. Pairwise comparisons among multiple groups were conducted using the LSD-*t* test. Survival curves were generated using the log-rank test and Kaplan-Meier technique, and prognosis was analyzed by multivariate Cox regression with multiple confounding factors. A p-value < 0.05 was considered statistically significant.

Results

The Expression Level of Hsa_circ_0120175 is Prominently Upregulated in the OC Tissues and Cell Lines

In this study, we analyzed ribosomal RNA-depleted total RNA in 3 OC tissues and 3 normal ovarian tissues using RNA-seq. We found a significantly upregulated circRNA, *hsa_circ_0120175* (Fig. 1A). To verify the loop formation and determine the cyclization site of *hsa_circ_0120175*, we subjected the PCR product of *hsa_circ_0120175* to Sanger sequencing, which confirmed the cyclization site (Fig. 1B). As the cellular compartment of *hsa_circ_0120175* was unknown, we performed nucleo-cytoplasmic separation experiments, which showed that the majority of *hsa_circ_0120175* was located in the cytoplasm ($p < 0.001$) (Fig. 1C).

Hsa_circ_0120175 was found to be elevated in OC tissues in three cases of the discovery cohort using RNA-seq. The expression level of *hsa_circ_0120175* was then verified by qRT-PCR in 40 OC tissues, 40 normal ovarian tissues, and three cell lines (one normal ovarian cell line and two different OC cell lines). The results showed that *hsa_circ_0120175* expression was significantly higher in OC tissues than in normal ovarian tissues ($p < 0.01$) (Fig. 1D). When compared with the normal ovarian cell line IOSE80, *hsa_circ_0120175* was considerably overexpressed in two OC cell lines (SK0V3 and HO8910) ($p < 0.001$) (Fig. 1E). These findings suggest a potential link between *hsa_circ_0120175* and OC.

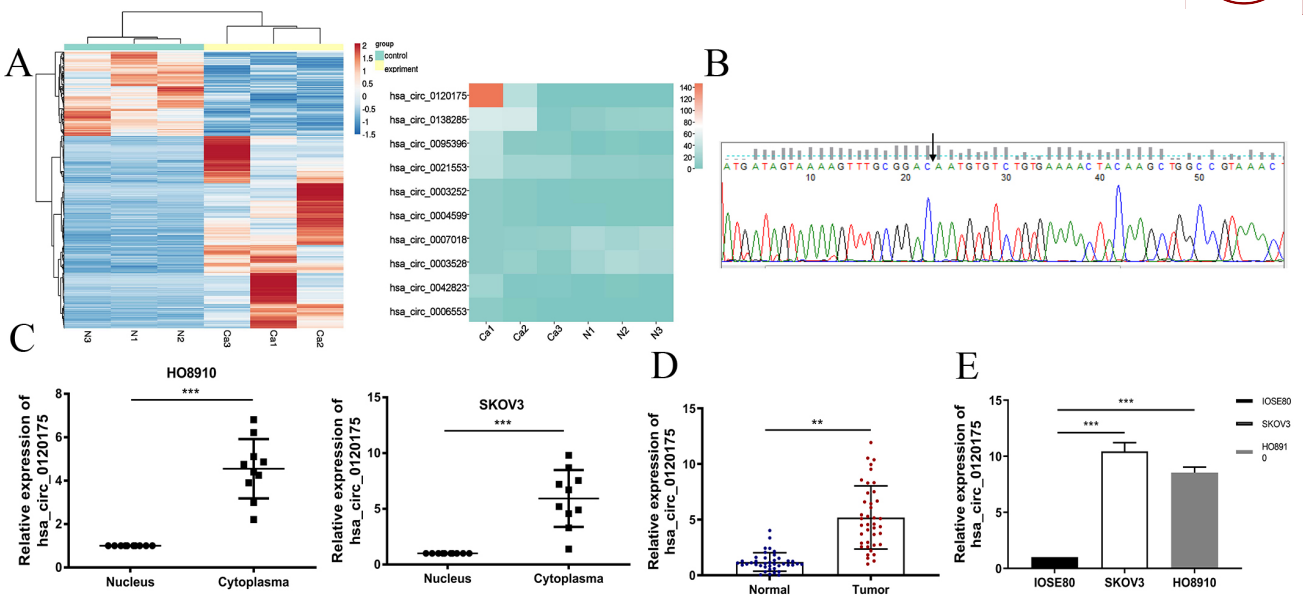

Fig. 1. Expression level of *hsa_circ_0120175* in OC tissues and cell lines. (A) Clustered heat map of the differentially expressed circRNAs in three normal ovarian tissues and three ovarian cancer tissues. Rows represent circRNAs; columns represent ovarian tissues. The circRNAs were classified based on the Pearson correlation. (B) Sanger sequencing of *hsa_circ_0120175* products amplified by PCR. The Cyclization site is marked by the black arrow. (C) Verification of the cytoplasmic localization of *hsa_circ_0120175* in OC cells by nuclear-cytoplasmic fractionation. (D) The expression levels of *hsa_circ_0120175* in OC tissues and normal ovarian tissues. (E) *Hsa_circ_0120175* expression levels in two human OC cell lines (SKOV3 and HO8910) and one normal human ovarian cell line (IOSE80). $^{**}p < 0.01$; $^{***}p < 0.001$. OC, ovarian carcinoma; circRNAs, circular RNAs.

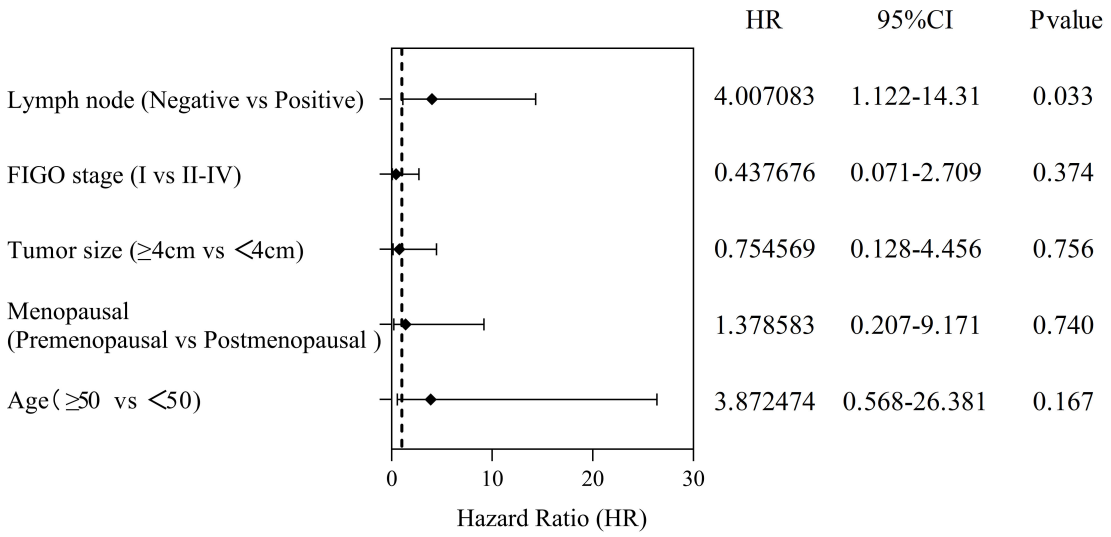

Fig. 2. Multivariate Cox regression analysis for overall survival. FIGO, The International Federation of Gynecology and Obstetrics.

Hsa_circ_0120175 Upregulation is Correlated with the Clinicopathological Parameters in OC

In this study, we investigated the relationship between the expression level of *hsa_circ_0120175* and clinicopathological features in OC patients. Patients were divided into two groups based on the median expression level of *hsa_circ_0120175* in OC tissues: low expression (<median) and high expression (≥median), as shown in Table 1. Our results showed that *hsa_circ_0120175* expression was significantly associated with The International Federation of Gynecology and Obstetrics (FIGO) stage, but not significantly correlated with tumor size, grade and lymph node metastasis (Table 1). Multivariate Cox regression analysis indicated that lymph node metastasis was closely related to prognosis, and the difference was statistically significant ($p < 0.05$) (Fig. 2). Furthermore, Kaplan-

Table 1. The expression level of *hsa_circ_0120175* and clinicopathological characteristics of OC patients.

Clinicopathological parameters		Expression level of *hsa_circ_0120175*			
Variables	Total (n = 40)	High (n = 20)	Low (n = 20)		*p*-value
Age				0.417	0.519
<50	16 (40%)	9 (22.5%)	7 (17.5%)		
≥50	24 (60%)	11 (27.5%)	13 (32.5%)		
Menopausal				0.102	0.749
Premenopausal	17 (42.5%)	8 (20%)	9 (22.5%)		
Postmenopausal	23 (57.5%)	12 (30%)	11 (27.5%)		
Tumor size (cm)				0.107	0.744
<4	15 (37.5%)	7 (17.5%)	8 (20%)		
≥4	25 (62.5%)	13 (32.5%)	12 (30%)		
Tumor grade				0.293	0.588
Higher	16 (40%)	9 (22.5%)	7 (17.5%)		
Moderately	13 (32.5%)	6 (15%)	7 (17.5%)		
Lower	11 (27.5%)	5 (12.5%)	6 (15%)		
FIGO stage				4.329	0.037*
I	7 (17.5%)	1 (2.5%)	6 (15%)		
II–IV	33 (82.5%)	19 (47.5%)	14 (35%)		
Lymph node metastasis				1.905	0.301
Negative	28 (70%)	16 (40%)	12 (30%)		
Positive	12 (30%)	4 (10%)	8 (20%)		

*$p < 0.05$. FIGO, The International Federation of Gynecology and Obstetrics.

Meier survival curves revealed that OC patients with high levels of *hsa_circ_0120175* expression had a low survival rate ($p < 0.05$) (Fig. 3).

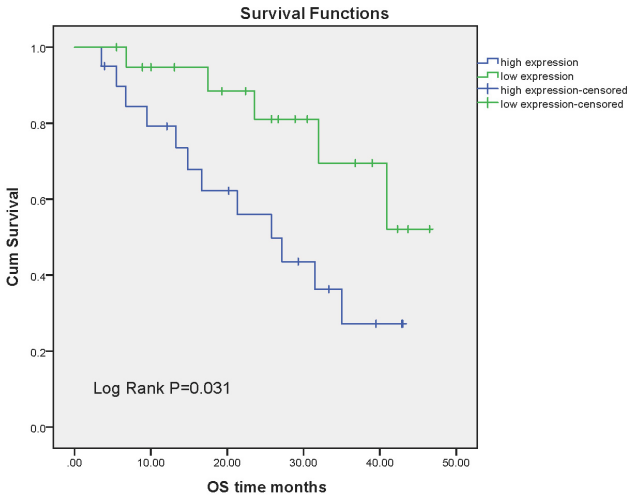

Fig. 3. The relationship between *hsa_circ_0120175* expression level and overall survival for OC. Kaplan-Meier survival analysis for OC patients with high (n = 20) versus low (n = 20) expression level of *hsa_circ_0120175* was conducted (Kaplan-Meier method and log-rank test; $p = 0.031$). OS, Overall Survival; Cum Survival, Cumulative Survival.

Biological Functions of Hsa_circ_0120175 in OC Cells

To investigate the biological effects of *hsa_circ_0120175*, we transfected the OC cell lines HO8910 and SKOV3 with siRNA to knock down the expression of *hsa_circ_0120175*. We then used qRT-RCR to detect the expression of *hsa_circ_0120175* in OC and confirm the silencing. The results showed that the expression levels of *hsa_circ_0120175* were lower in the si-circRNA group (*hsa_circ_0120175* in OC cells with siRNA silencing) than in the blank control group and the si-NC group (Fig. 4A). There were no statistically significant differences between the blank control group and the si-NC group ($p < 0.01$) (Fig. 4A). The colony formation test, CCK-8 assay, Transwell® assay, and wound healing assay were then used to evaluate OC cell proliferation, migration, and invasion. The results showed that silencing *hsa_circ_0120175* significantly decreased OC cell growth in the CCK-8 test and wound healing assay ($p < 0.01$) (Fig. 4B,C). Additionally, the Transwell® and Matrigel Transwell® experiments revealed that silencing *hsa_circ_0120175* significantly reduced cell migration and invasion in H08910 and SKOV3 cells ($p < 0.01$) (Fig. 4D–G). The wound healing ability of *hsa_circ_0120175* silenced cells was much inferior to that of si-NC control cells in the wound healing experiment ($p < 0.01$) (Fig. 4H,I). Overall, the study suggests that *hsa_circ_0120175* enhances OC cell proliferation, migration, and invasion *in vitro*.

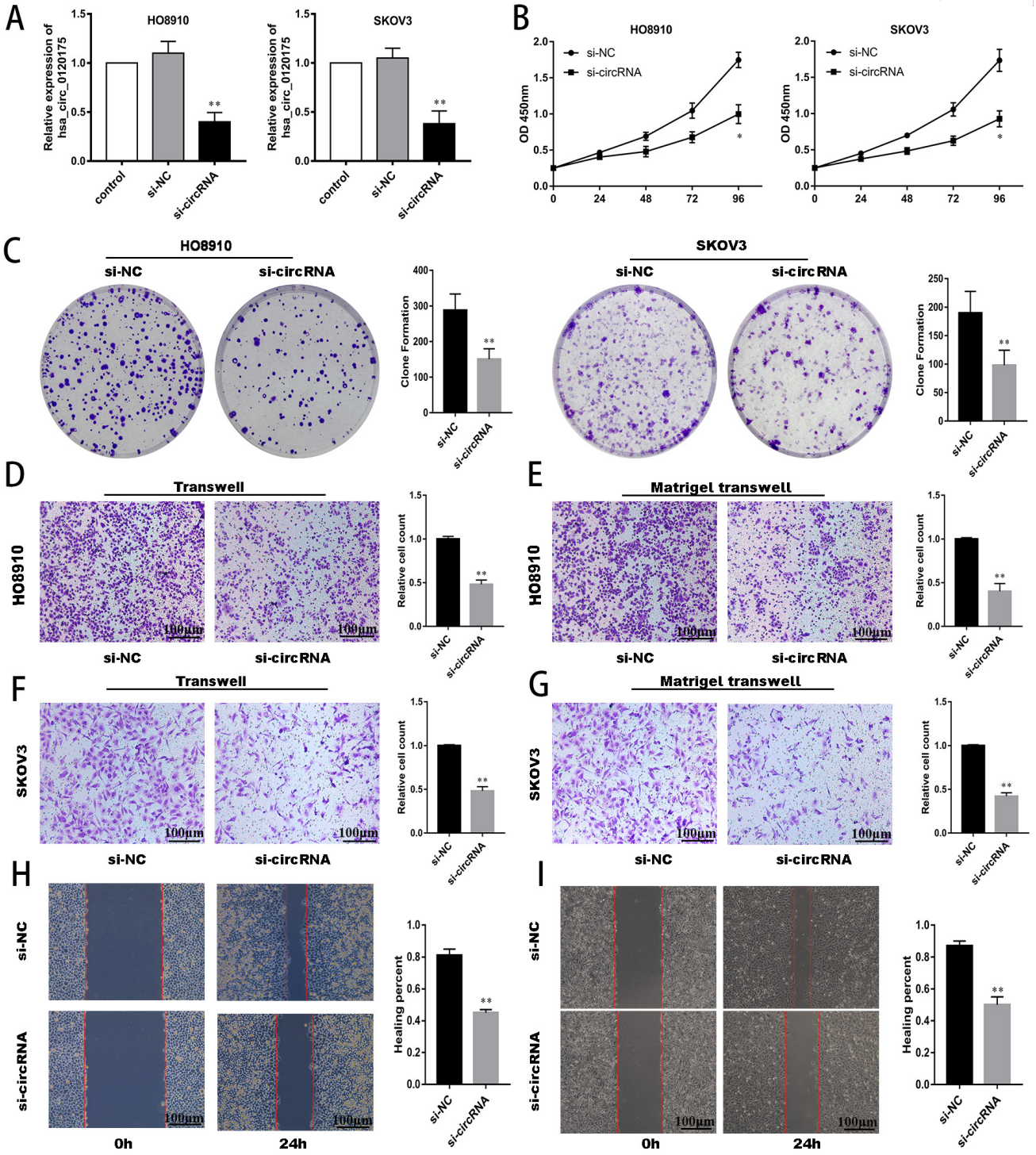

Fig. 4. Biological function of *hsa_circ_0120175* in OC cells. (A) Expression levels of *hsa_circ_0120175* in OC cells with siRNA silencing (si-circRNA group) detected by qPCR, compared with negative control siRNA (si-NC) or blank control. (B,C) Cell Counting Kit-8 (CCK-8) assays (B) and colony formation assays (C) show the decreased cell proliferation of HO8910 and SKOV3 cells with *hsa_circ_0120175* silencing (si-circRNA), compared with the negative control siRNA (si-NC). (D–I) The effect of *hsa_circ_0120175* on the migration and invasion ability of OC cells with *hsa_circ_0120175* silencing (si-circRNA), compared with negative control siRNA (si-NC), was evaluated by Transwell® migration (D,F), Matrigel invasion (E,G), and wound healing assays (H,I). $*p < 0.05$, $**p < 0.01$.

Discussion

Recent fast advancements in bioinformatics and high-throughput sequencing have led to an increased interest in circRNAs, which are being studied in clinical trials [15,16]. CircRNA has great potential as a molecular biomarker of disease due to its structural properties that lack the 5′ cap

and 3′ poly(A) tail, making it less susceptible to degradation by ribonucleases and conferring good stability compared to linear RNA [17,18]. OC is a fatal disease that starts insidiously, metastasizes early, and recurs easily after treatment [19]. Epithelial OC is the most frequent type and has a very poor prognosis [20]. Despite recent breakthroughs in OC treatment options, the overall survival of OC patients has not improved notably, and the prognosis of OC patients remains uncertain [21].

In the existing OC-related studies, only a small number of circRNAs have been confirmed to have an impact on the initiation and development of OC. However, the majority of circRNAs in OC have not yet been researched [22,23].

In this study, we discovered for the first time that the expression level of *hsa_circ_0120175* was significantly elevated in OC tissues. Circbase is a database for circular RNAs. Data from various *Homo sapiens*, *Caenorhabditis elegans*, *Mus musculus*, and *Latimeria* samples have been included in this database. Based on Circbase, we knew that *hsa_circ_0120175* was located on chromosome 2 (chr2: 47,600,601–47,602,438) and was derived from the EPCAM gene [24]. However, *hsa_circ_0120175* had never been reported in tumors of any type, including human OC. We found that *hsa_circ_0120175* expression level was dramatically upregulated in OC tissues first by RNA sequencing in three OC tissues of a discovery cohort (unpublished data), and then by qRT-PCR validation in a large cohort of OC tissues and OC cell lines.

In this study, we investigated the link between *hsa_circ_0120175* expression and clinicopathological characteristics, as well as anticipated survival time, in OC patients. Our findings revealed that the expression levels of *hsa_circ_0120175* were associated with FIGO stage. Furthermore, we observed that patients with high expression levels of *hsa_circ_0120175* had a poorer prognosis and shorter survival time compared to those with low expression levels. These results suggest that *hsa_circ_0120175* may play a vital role in the progression of OC.

We conducted several assays to investigate the biological activities of *hsa_circ_0120175* in OC cells by reducing its expression in HO8910 and SKOV3 cells. The assays included colony formation, CCK-8, Transwell®, and wound healing assays. The results showed that *hsa_circ_0120175* knockdown significantly decreased the rates of cell proliferation, migration, and invasion in these two OC cell lines. This finding is consistent with the fact that *hsa_circ_0120175* has a carcinogenic impact in OC and is associated with a poor prognosis in OC patients.

Conclusions

In this study, it was found for the first time that *hsa_circ_0120175* is an oncogene that promotes the development of OC. The high expression of *hsa_circ_0120175* may boost OC cell proliferation, migration, and invasion *in vitro*. Further research may lead to *hsa_circ_0120175* becoming an underlying tumor biomarker for OC diagnosis as well as a novel target for OC therapy.

Availability of Data and Materials

All data included in this study are available upon request by contact with the corresponding author.

Author Contributions

YL, SL, TX contributed to the conception of the study; TX, JD, JL performed the experiment; TX, JC contributed significantly to analysis and manuscript preparation; TX, YL and SL performed the data analyses and wrote the manuscript; JC, JD, YL helped perform the analysis with constructive discussions. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Our research was carried out after the Ethical Committee of Wannan Medical College's First Affiliated Hospital approved it, and informed consent was obtained from all participants (the name of the ethics committee: 2022 Lun Review Research No. 04). The human studies in this study comply with the Declaration of Helsinki.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Li C, Wang Y, Wang H, Wang B, Wang Y, Li N, *et al*. miR-486 Promotes the Invasion and Cell Cycle Progression of Ovarian

Cancer Cells by Targeting CADM1. Analytical Cellular Pathology. 2021; 2021: 7407086.

[2] Stewart C, Ralyea C, Lockwood S. Ovarian Cancer: An Integrated Review. Seminars in Oncology Nursing. 2019; 35: 151–156.

[3] Arentz G, Mittal P, Klingler-Hoffmann M, Condina MR, Ricciardelli C, Lokman NA, *et al*. Label-Free Quantification Mass Spectrometry Identifies Protein Markers of Chemotherapy Response in High-Grade Serous Ovarian Cancer. Cancers. 2023; 15: 2172.

[4] Scaletta G, Plotti F, Luvero D, Capriglione S, Montera R, Miranda A, *et al*. The role of novel biomarker HE4 in the diagnosis, prognosis and follow-up of ovarian cancer: a systematic review. Expert Review of Anticancer Therapy. 2017; 17: 827–839.

[5] Zhang XW, Wu YS, Xu TM, Cui MH. CAR-T Cells in the Treatment of Ovarian Cancer: A Promising Cell Therapy. Biomolecules. 2023; 13: 465.

[6] Sanger HL, Klotz G, Riesner D, Gross HJ, Kleinschmidt AK. Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. Proceedings of the National Academy of Sciences of the United States of America. 1976; 73: 3852–3856.

[7] Vo JN, Cieslik M, Zhang Y, Shukla S, Xiao L, Zhang Y, *et al*. The Landscape of Circular RNA in Cancer. Cell. 2019; 176: 869–881.e13.

[8] Mo M, Liu B, Luo Y, Tan JHJ, Zeng X, Zeng X, *et al*. Construction and Comprehensive Analysis of a circRNA-miRNA-mRNA Regulatory Network to Reveal the Pathogenesis of Hepatocellular Carcinoma. Frontiers in Molecular Biosciences. 2022; 9: 801478.

[9] Li Y, Ge YZ, Xu L, Jia R. Circular RNA ITCH: A novel tumor suppressor in multiple cancers. Life Sciences. 2020; 254: 117176.

[10] Ye X, Zhu B, Han J, Huang J, Wu Y. Circ-0036602 Acts As a Sponge of MiR-34a-5p and MiR-431-5p to Promote Cervical Cancer Proliferation and Invasion. Journal of Genomics. 2022; 10: 16–25.

[11] Wang X, Ji C, Hu J, Deng X, Zheng W, Yu Y, *et al*. Hsa_circ_0005273 facilitates breast cancer tumorigenesis by regulating YAP1-hippo signaling pathway. Journal of Experimental & Clinical Cancer Research. 2021; 40: 29.

[12] Zhang S, Cheng J, Quan C, Wen H, Feng Z, Hu Q, *et al*. circCELSR1 (hsa_circ_0063809) Contributes to Paclitaxel Resistance of Ovarian Cancer Cells by Regulating FOXR2 Expression via miR-1252. Molecular Therapy. Nucleic Acids. 2020; 19: 718–730.

[13] Shen C, Wu Z, Wang Y, Gao S, Da L, Xie L, *et al*. Downregulated hsa_circ_0077837 and hsa_circ_0004826, facilitate bladder cancer progression and predict poor prognosis for bladder cancer patients. Cancer Medicine. 2020; 9: 3885–3903.

[14] Kong S, Yang Q, Tang C, Wang T, Shen X, Ju S. Identification of hsa_circ_0001821 as a Novel Diagnostic Biomarker in Gastric Cancer *via* Comprehensive Circular RNA Profiling. Frontiers in Genetics. 2019; 10: 878.

[15] Geng Y, Jiang J, Wu C. Function and clinical significance of circRNAs in solid tumors. Journal of Hematology & Oncology. 2018; 11: 98.

[16] Zheng Y, Hui T, Yue C, Sun J, Guo D, Guo S, *et al*. Comprehensive analysis of circRNAs from cashmere goat skin by next generation RNA sequencing (RNA-seq). Scientific Reports. 2020; 10: 516.

[17] Shafabakhsh R, Mirhosseini N, Chaichian S, Moazzami B, Mahdizadeh Z, Asemi Z. Could circRNA be a new biomarker for pre-eclampsia? Molecular Reproduction and Development. 2019; 86: 1773–1780.

[18] Sole C, Arnaiz E, Manterola L, Otaegui D, Lawrie CH. The circulating transcriptome as a source of cancer liquid biopsy biomarkers. Seminars in Cancer Biology. 2019; 58: 100–108.

[19] Wang Q, Zhi Z, Han H, Zhao Q, Wang X, Cao S, *et al*. Statin use improves the prognosis of ovarian cancer: An updated and comprehensive meta-analysis. Oncology Letters. 2022; 25: 65.

[20] Wu J, Wu Y, Guo Q, Wang S, Wu X. RNA-binding proteins in ovarian cancer: a novel avenue of their roles in diagnosis and treatment. Journal of Translational Medicine. 2022; 20: 37.

[21] Zhang SZ, Xie L, Shang ZJ. Burden of Oral Cancer on the 10 Most Populous Countries from 1990 to 2019: Estimates from the Global Burden of Disease Study 2019. International Journal of Environmental Research and Public Health. 2022; 19: 875.

[22] Zhang L, Zhou Q, Qiu Q, Hou L, Wu M, Li J, *et al*. CircPLEKHM3 acts as a tumor suppressor through regulation of the miR-9/BRCA1/DNAJB6/KLF4/AKT1 axis in ovarian cancer. Molecular Cancer. 2019; 18: 144.

[23] Zong ZH, Du YP, Guan X, Chen S, Zhao Y. CircWHSC1 promotes ovarian cancer progression by regulating MUC1 and hTERT through sponging miR-145 and miR-1182. Journal of Experimental & Clinical Cancer Research. 2019; 38: 437.

[24] Glažar P, Papavasileiou P, Rajewsky N. circBase: a database for circular RNAs. RNA. 2014; 20: 1666–1670.