OCT4 Promotes Ovarian Cancer Cell Metastasis and Angiogenesis via Modulating VEGFR2/LRPPRC Pathway

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Research

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Abstract

Background: Due to its high ability of metastasis, ovarian cancer remains the most lethal gynecological malignancy, yet its underlying mechanism remains unconfirmed.

Objectives: The main purpose is to probe into the role and regulation mechanism of octamer-binding transcription factor 4 (OCT4) in angiogenesis and metastasis in ovarian cancer.

Methods: Immunohistochemistry (IHC) and immunofluorescence in epithelial ovarian cancer specimens and benign ovarian tumor samples were performed, followed by RNA-sequencing and examination of angiogenesis, cell migration and invasion in OCT4 knockdown cell lines and the controls. Co-Immunoprecipitation (Co-IP), mass spectrometry, immunoblotting, immunofluorescence and chromatin immunoprecipitation (ChIP) analyses were conducted along with models of zebrafishes and nude mice of transplanted tumors to gain insights into the specific functions and mechanisms of action of OCT4 in ovarian cancer.

Results: Firstly, we discovered that OCT4 expression was enhanced in ovarian cancer tissues significantly, especially in the metastatic lesions, indicating that OCT4 might be a key for the metastasis of ovarian cancer. Furtherly, we observed and verified that OCT4 promoted cell migration and invasion, and induced angiogenesis in vitro and in vivo. Mechanistically, OCT4 modulated the transcription of leucine-rich PPR motif-containing protein (LRPPRC) and furtherly interacted with vascular endothelial growth factor receptor 2 (VEGFR2)/LRPPRC complex and ultimately triggered the downstream FAK/AKT signaling pathway.

Conclusions: Together, through models of ovarian cancer cells, zebrafishes and tumor-transplanted mice, this study highlighted the importance of OCT4/LRPPRC/VEGFR2 signaling axis in metastasis of ovarian cancer and angiogenesis. Thus, our finding supplied a potential novel molecular-targeted approach for the treatment of ovarian cancer.

Introduction

With metastatic state of 70% patients at diagnosis, ovarian cancer remains the gynecological malignancy with highest mortality, though there were progression in the therapeutic modes, including surgery and chemotherapy[1, 2]. As is reported, the 5-year survival rate of ovarian cancer patients is only 30.2% [3]. However, the mechanism of action of ovarian cancer progression and metastasis remains uncertain. The objective of our study is to discover key factors participating in cell metastasis and probe into the molecular mechanism.

Octamer-binding transcription factor 4 (OCT4), also named as pit-oct-unc (POU) domain class 5 transcription factor 1 (POU5F1), is a noted regulatory factor of self-renewal and cell differentiation in stem cells [4, 5]. It was first reported in 1989 [6], and its expression was discovered in ovulated oocytes, inner cell mass, embryonic stem cells, embryonic germ cells, and embryonic carcinoma cells [7].
Nowadays, OCT4 has been detected in several sorts of human cancers and observed to play an essential role in carcinogenesis. For example, OCT4 was enhanced in breast cancer[8], colorectal cancer[9, 10], liver cancer[11], and ovarian cancer[12]. Moreover, OCT4 could promote prostate cancer progression[13] and liver metastasis of primary colorectal cancer [9], and accelerate tumorigenesis through activating JAK/STAT signaling in ovarian cancer [14]. However, function of OCT4 in the metastasis of ovarian cancer and its underlying molecular mechanisms remain uncertain.

Firstly, our study revealed that OCT4 was up-regulated in epithelial ovarian cancer patients compared with the benign ovarian tumors, especially in the metastatic lesions. Functionally, OCT4 promoted angiogenesis and ovarian cancer cell metastasis in \textit{vitro} and in \textit{vivo}. Mechanistically, OCT4 induced the transcription of leucinerich PPR motifcontaining protein (LRPPRC), and furtherly interacted with vascular endothelial growth factor receptor 2 (VEGFR2)/LRPPRC complex, and ultimately triggered its downstream FAK/AKT pathway. Accordingly, we proposed that OCT4 played a hitherto unrecognized mechanism in metastasis of ovarian cancer, and the novel OCT4/LRPPRC/VEGFR2 signaling axis might be effectively used for a promising therapeutic target.

\textbf{Results}

\textbf{OCT4 was markedly enhanced in metastatic ovarian cancer tissues and might serve as an oncogene to lead to poor survival in the patients}

Firstly, we constructed 8 three-dimensional (3D) cultured organoids of epithelial ovarian cancer and performed immunofluorescence, and ultimately discovered that OCT4 was high expressed in ovarian cancer organoids (Fig. 1A). Then, via immunofluorescence staining assay in 20 primary ovarian cancer tissues and 20 metastatic specimens from greater omenta, and we discovered that the metastatic specimens exhibited higher expression of OCT4 compared with the primary lesions (Fig. 1B). Furtherly, we established tissue chips using 153 benign ovarian tumor tissues, 187 primary lesions of ovarian cancer, and 154 metastatic specimens from greater omenta. Through immunohistochemistry (IHC) technique and image analysis of these chips we discovered that OCT4 was high expressed in ovarian cancer tissues, especially in the metastatic sites (Fig. 1C). H-score of IHC evaluation was shown in Fig. 1D, and the statistics demonstrated that OCT4 high expression ration in metastatic lesions of ovarian cancer was obviously higher than that in primary specimens (81.82% vs. 63.64%), and both of them were higher than benign ovarian tumor tissues (18.95%, Fig. 1E). Furthermore, results of survival analysis showed that OCT4 high expression was significantly associated with shorter survival (OS and PFS) in ovarian cancer patients (Fig. 1F-G). Together, above results demonstrated that OCT4 might serve as an oncogene to participate in ovarian cancer metastasis, and an enhanced OCT4 induced ovarian cancer patients' poor prognosis.

\textbf{OCT4 elevated cell migration and invasion in ovarian cancer}
To further probe into the modulation role of OCT4 in ovarian cancer, we established two kinds of OCT4-targeting shRNAs and the control vector into ovarian cancer cell lines (OCT4-silencing ovarian cancer cell lines: SKOV3ip1/OCT4-sh1/2 and OVCA433/OCT4-sh1/2; control ovarian cancer cell lines: SKOV3ip1/Vector and OVCA433/Vector), which was proved by qRT-PCR and immunoblotting assay (Fig. 2A-B). Next, we performed RNA-sequencing in SKOV3ip1/OCT4-sh1 ovarian cancer cells and the controls, and found that a number of differentially expressed genes (DEGs) were significantly altered (Fig. 2C). Gene ontology (GO) analysis of biological process indicated that the DEGs were closely associated with cell migration and angiogenesis (Fig. 2D). Meanwhile, KEGG pathway analysis showed that DEGs were enriched in VEGF pathways and some pathways in cancer (Fig. 2E).

Following Gene set enrichment analysis (GSEA) analysis displayed that cell migration related genes were depleted in OCT4 silencing ovarian cancer cells significantly (Fig. 3A). Immunoblotting assay demonstrated that metastasis-inducing protein N-cadherin was decreased and metastasis restrained protein E-cadherin was increased obviously (Fig. 3B). Subsequently, decreased migration range was discovered via wound healing assay. The migration areas of SKOV3ip1/OCT4-sh1 and SKOV3ip1/OCT4-sh2 cells were lessened by 62.43% and 54.60%; and that of SKOV3ip1/OCT4-sh1 and SKOV3ip1/OCT4-sh2 cells were decreased by 48.73% and 47.82%, compared with their controls, respectively (Fig. 3C, 3F). Transwell assays of migration and invasion were also conducted. We identified that the count of migrated cells was abated by 66.94% and 59.63% in SKOV3ip1/OCT4-sh1 and SKOV3ip1/OCT4-sh2 ovarian cancer cells and by 40.93% and 35.47% in OVCA433/OCT4-sh1 and OVCA433/OCT4-sh2 cells, compared with controls, respectively (Fig. 3D, 3G). Count of invaded cells was decreased by 57.30% and 43.06% in SKOV3ip1/OCT4-sh1 and SKOV3ip1/OCT4-sh2 ovarian cancer cells and by 46.04% and 40.85% in OVCA433/OCT4-sh1 and OVCA433/OCT4-sh2 cells, respectively, compared with their controls (Fig. 3E, 3H). Therefore, it can be concluded that OCT4 promoted cell migration and invasion in ovarian cancer.

**OCT4 induced angiogenesis and ovarian cancer cell metastasis via VEGFR2-LRPPRC signaling axis**

Through above Fig. 2D-E we inferred that OCT4 was associated with angiogenesis in ovarian cancer. Moreover, GSEA analysis of the RNA-sequencing showed that OCT4 knockdown significantly restrained vascular generation (Fig. 4A). To verify the assumption, we performed tube formation assay in human umbilical vein endothelial cells (HUVECs), which indicating that when HUVECs were cultured by ovarian cancer cells’ supernatant from SKOV3ip1/OCT4-sh1, SKOV3ip1/OCT4-sh2, OVCA433/OCT4-sh1 and OVCA433/OCT4-sh2 cells, the tube formation was greatly inhibited by OCT4 silencing (Fig. 4B-C). As is well known, VEGFR2 is a crucial regulator and receptor in regulation of angiogenesis in VEGF pathway among a number of tumor vasculature-associated molecules. To probe into the interacting proteins of VEGFR2, immunoprecipitationi (IP) assay and mass spectrum (MS) analyses were performed, and 92 binding proteins were found (**Supplemental table 2**). Many of those proteins were cytoskeleton proteins, and finally we chose an interesting protein named LRPPRC for further study (Fig. 4D-E). And then, the
interaction between VEGFR2 and LRPPRC was furtherly verified by Co-IP (Fig. 4F) and immunofluorescence (Fig. 4G).

Interestingly, via websites of Jaspar and NCBI we predicted that the transcription factor OCT4 might bind to upstream of LRPPRC promoter region. Firstly, we performed qRT-PCR assay and subsequently found that OCT4 knockdown decreased mRNA expression level of LRPPRC significantly (Fig. 5A). Next, we verified that the protein expression level of LRPPRC was also available decreased by OCT4 silencing via immunoblotting and immunofluorescence (Fig. 5B-C). Subsequently, we conducted ChIP assay to deeply explore the association between OCT4 and LRPPRC, and we concluded that OCT4 bound to specific DNA-binding sites to induce LRPPRC transcription in the upstream region of transcription start site (TSS) (Fig. 5D). In the following study, we established LRPPRC silencing cell lines and observed that phosphorylation activation of VEGFR2 was inhibited by LRPPRC knockdown (Supplementary Fig. 1A). A series of subsequent functional experiments demonstrated that LRPPRC silencing restricted vascular formation, and cell migration and invasion (Supplementary Fig. 1B-E). Through subsequent rescue experiment, we identified that LRPPRC overexpression effectively reversed the inhibition of tube formation, and ovarian cancer cell migration and invasion induced by OCT4 reduction (Supplementary Fig. 2A-E). Following study verified that OCT4 silencing inhibited VEGFR2 phosphorylation activation at the sites of Tyr1175 and Tyr1214 (Fig. 5E, Supplementary Fig. 3A-B), and significantly altered the downstream pathway proteins such as AKT1 and many other molecules associated with angiogenesis and cell metastasis (Fig. 5E-F).

In general, we concluded that OCT4 induced angiogenesis and promoted cell migration and invasion via VEGFR2/LRPPRC pathway at the transcription level.

**OCT4 activates vascular formation and promotes ovarian cancer progression and metastasis *in vivo***

In order to verify the above standpoints, subcutaneous and intraperitoneal nude mice models of ovarian cancer were generated. We investigated that xenograft tumor growth was restrained by OCT4 knockdown markedly, which was increased by the overexpression of LRPPRC significantly (Fig. 6A-F). After the mice in the control group of intraperitoneal xenograft model were sacrificed and dissected, we tested the association between OCT4 and LRPPRC via immunofluorescence, and verified that OCT4 silencing reduced LRPPRC expression in tumor tissues of ovaries (Fig. 6G). Then, we demonstrated that OCT4 silencing decreased the expression of vascular endothelial cell-specific marker CD31 by immunofluorescence too (Fig. 6H). In our study, we constructed zebrafish models to detect angiogenesis *in vivo*. OCT4 morphlino suppressed zebrafish vascular formation, and the inhibitory action was turned back by overexpression of LRPPRC (Fig. 6I). Together, the findings suggested that OCT4 acted as an oncogene and promoted angiogenesis and ovarian cancer metastasis *in vivo*.

**The expression and association among OCT4, LRPPRC and VEGFR2 in human ovarian cancer specimens**
To detect the expression of LRPPRC, we performed IHC assay and observed that LRPPRC was substantially upregulated in human epithelial ovarian cancer samples, especially in metastatic lesions, compared with benign ovarian tumors (Fig. 7A). And then survival analysis demonstrated that high LRPPRC was correlated with poor OS and PFS in ovarian cancer patients (Fig. 7B). The positive ratio of LRPPRC in metastatic lesions of human ovarian cancer was 85.71%, which was obviously higher compared with the primary ovarian cancer specimens (66.84%), and both of them were higher than benign ovarian tumor tissues (38.56%, Fig. 7C). Following immunofluorescence assay indicated that OCT4 and angiogenesis biomarker CD31 were greatly high expressed in ovarian cancer metastatic lesions (Fig. 7D). Additionally, via qRT-PCR we discovered that relative mRNA levels of angiogenesis and metastasis-related proteins such as VEGFA, AKT1 and N-cadherin were upregulated in OCT4 high expressed ovarian cancer tissues, and simultaneously E-cadherin was markedly reduced (Fig. 7E). Ultimately, we summarized the mechanism in this study and drew the novel pathway of OCT4/VEGFR2/LRPPRC in ovarian cancer (Fig. 7F).

**Discussion**

Our study clarified a new molecular mechanism of metastasis in ovarian cancer. Briefly, initial immunohistochemistry and immunofluorescence analyses disclosed that OCT4 was significantly enhanced in ovarian cancer metastatic lesions; subsequently, OCT4 induced angiogenesis and ovarian cancer cell metastasis via VEGFR2/LRPPRC axis. Thus, our findings highlight the role of OCT4/LRPPRC/VEGFR2 axis in metastasis of ovarian cancer, which supplied promising therapeutic targets for ovarian cancer.

OCT4, essential for the maintenance of stem cell phenotypes and pluripotent characters[15], has been verified to be indispensable for cancer cell stemness [16], and discovered to boost oncogenesis [17, 18]. OCT4 was reported to be remarkably high expressed in lung adenocarcinoma[19], colorectal cancer[20], cervical cancer[21], ovarian cancer[22], and so on. Our finding indicated that OCT4 could activate angiogenesis and promote ovarian cancer metastasis by regulating LRPPRC/ VEGFR2 axis.

LRPPRC belongs to the PPR motif-containing proteins family, which can regulate transcription and the process of translation[23]. It was shown that gene mutation of LRPPRC led to Leigh syndrome French–Canadian[24]. Nowadays, more and more studies have demonstrated that LRPPRC dysregulation is associated with tumors. LRPPRC expression was increased in various tumor cell lines and cancer tissues, including colorectal cancer[25],gastric cancer[26], prostate cancer[27, 28], and so on. Moreover, LRPPRC plays an important function in the progression of some cancers and predicts poor prognosis[29], and the restriction presents a promising potential molecular strategy for the tumor therapy [28]. Current studies about signal pathways of LRPPRC are limited, and LRPPRC has been seldom reported in ovarian cancer. Our study demonstrated that LRPPRC was high expressed in ovarian cancer, especially in the metastatic lesions, and LRPPRC could bind to VEGFR2 to activate it to promote angiogenesis and metastasis of ovarian cancer cells finally.
Angiogenesis participates in multiple processes of tumor behavior, including metabolism[30], metastasis processes[31], and cancer stem cell maintenance[32, 33]. Vasculature is largely quiescent in the normal conditions [34], but an “angiogenic switch” is usually aroused in tumors by several angiogenic factors resulting in vascular formation [35]. Nowadays, antiangiogenic treatments have shown efficacy for some malignancies such as colorectal cancer[36, 37], epithelial ovarian cancer[38, 39] and non-small-cell lung cancer [40, 41]. Interestingly, we proved that OCT4 knockout inhibited angiogenesis via inhibiting VEGFR2/LRPPRC pathway. Then, we infer that the OCT4/LRPPRC/VEGFR2 axis may be an important therapeutic target in ovarian cancer, though it demands further clinical verification.

**Conclusion**

On the whole, our finding supplied a novel mechanism of action of metastasis in ovarian cancer, and provided a potential antiangiogenic strategy via targeting OCT4/LRPPRC/VEGFR2 signaling pathway.

**Materials And Methods**

**Tissue specimens**

In our study, 153 benign ovarian tumor tissues, 187 primary lesions of epithelial ovarian cancer, and 154 metastatic specimens from greater omenta were collected after acquisition of the informed consent in Xinhua Hospital (Shanghai, China). Tissue samples were gained between 2008 and 2017. Overall survival (OS) was calculated as the interval from surgery initiation to death for whatever reason or final time for follow-up. Progression-free survival (PFS) was measured from surgery initiation to disease relapse or progression.

**Ovarian cancer organoids generation**

Ovarian cancer organoids were gained from human high-grade serous ovarian carcinoma (HGSOC). Organoids generation and culture were performed as previously described[42]. Fresh cancer tissues were promptly carried to laboratory and dipped in Advanced Dulbecco's Modified Eagle's Medium (DMEM)/F12. Tissues were diced into approximately 2-3 mm sections and subsequently digested in 37°C for 1 hour.

**OCT4 knockdown cell lines construction and culture**

Ovarian cancer SKOV3ip1 and OVCA433 cells were cultured in DMEM with 10% FBS included (Gibco, Life Technologies). To generate stable cell lines, lentiviruses harboring scrambled shRNA (Vector) or two kinds of specific shRNAs against OCT4 (OCT4-sh1/2) were transduced into above ovarian cancer cells. The shRNA sequences of OCT4 were as follows: 5’- CACACCAGTTATCAATCTCCC-3’ (sh1); 5’- CCATTCGGGATTCAAGAACCT- 3’ (sh2). The silencing effect of OCT4 was confirmed by follow-up immunoblotting.
Wound healing assay

Firstly, cells were seeded on 6-well plates with $9 \times 10^5$ cells/well as confluent monolayers. Subsequently, scratch wound healing assay was performed by pipette tips. After incubating for 48 h, we calculated the migrated area using microscopy.

Transwell assays of migration and invasion

Transwell migration and invasion assays were performed as previously described[43].

$5 \times 10^4$ cells were planted into the upper chamber plates and cultured with serum-free DMEM medium. The lower chamber was added with DMEM containing 10% FBS. Non-migrated or non-invasive cells were removed by cotton buds after 48 h, and the migrated or invasive cells were stained with crystal violet. Stained cells were counted in five random fields per well under a light microscope (Nikon, Japan).

Tube formation

Firstly, 24-well plates were coated with 300 μL matrigel (BD Biosciences). Subsequently, $2 \times 10^4$ cells were suspended in 200 μL medium. 6 h later, we investigated and measured tube formation using a microscope (Olympus, Japan).

Immunoprecipitation

Pierce Crosslink Immunoprecipitation kit was utilized to perform immunoprecipitation (IP) following the manufacturer's protocol. In short, lysates of SKOV3ip1 ovarian cancer cells were generated via lysis buffer and then tested by BCA Protein Assay Kit (Beyotime, China). In the subsequent procedures, 1 mg total cell lysates were pre-cleared and blended with 10 μg antibody or IgG, and co-incubated with A/G agarose beads at 4°C overnight. Finally, immunoblotting assay was carried out to examine the target proteins.

Mass spectrometry

Firstly, cell sediment was collected by centrifugation. And then, protein was extracted by 8 mol/L urea lysate, which was dissolved in 100 mmol/L ammonium bicarbonate. Through Bicinchoninic Acid (BCA) assay, protein concentration was determined and 300 μg protein was used for the following mass spectrometry (MS) assay. After processes of reduction reaction, purification and enzymatic hydrolysis, samples were desalted via the desalination column, and finally tested by mass spectrometer (Thermo Fisher, USA).

Immunoblotting

After protein quantification via BCA assay 20 μg protein was utilized for immunoblotting assay. Subsequently, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed and proteins were transferred to polyvinylidene fluoride (PVDF) membrane. Next, primary
antibodies were added to the membrane and incubated for 2 hours at Room temperature (RT). In our study, primary antibodies against OCT4 (ab181557), LRPPRC (ab97505), p-VEGFR2 (Tyr1214, ab131241), VEGFR2 (CST 9698), p-VEGFR2 (Tyr1175, CST 2478), p-AKT (Ser473, CST 9018) and β-actin (CST3700) were used. Then, the membrane was washed for 3 times, and incubated with corresponding secondary antibodies. Finally, chemiluminescence kit (Millipore, USA) was applied for visualization of protein bands.

**RNA sequencing**

After total RNA was extracted by TRIzol (Thermo Fisher, USA), 1 mg RNA was used for subsequent RNA sequencing analysis. The method was detailed described as reported[44].

**Immunohistochemistry assay**

Paraffin-embedded tissue was sliced into 3 µm thin sections for immunohistochemistry (IHC) analysis. Antibodies of OCT4 (ab181557, Abcam) and LRPPRC (ab97505, Abcam) were applied and subsequently incubated with corresponding secondary antibodies at 37°C for 1 h. Histochemistry score (H-score) and positive ratio were utilized to assess the expression level as described formerly[43].

**Quantitative real time PCR**

Total RNA was centrifugated and extracted from ovarian cancer cells and tissues with TRIzol reagent (Invitrogen, US) in order to perform quantitative real time PCR (qRT-PCR). Primers in our study were exhibited in Supplemental Table 1.

**Immunofluorescence assay**

Ovarian cancer cells or tissues were fixed with 4% paraformaldehyde at room temperature (RT) for 15 min and then incubated with 0.3% Triton X-100. After blocking of 5% goat serum (Life Technologies, US) at RT, cells or tissues were treated with appropriate amount of antibody overnight at 4 °C. In the next step, primary antibody was cleared up by phosphate buffered saline (PBS) for 3 times, and then corresponding secondary antibody was added to the cells or tissues. 4′,6-diamidino-2-phenylindole (DAPI, Life Technologies, US) was utilized for nuclear staining. Ultimately, the stained cells were visualized using a Leica SP5 confocal fluorescence microscope.

**Nude mice model**

BALB/c nude mice tumor associated experiments protocols were authorized and approved by Ethics Committee at Xinhua hospital. In this study, a total of 60 female mice (aged 4 weeks) were used. The models of subcutaneous and intraperitoneal ovarian cancer mice were generated by injection of 5 × 10^6 SKOV3ip1 cells. Subcutaneous tumor volume was regularly measured and calculated twice a week by vernier caliper. Intraperitoneal tumor volume of the ovarian cancer mode was observed by luminescence imaging techniques.
Zebrafish model

In our study, transgenic enhanced green fluorescent protein (EGFP) expressing zebrafish model was used for assessing angiogenesis in vivo. OCT4 morpholino plasmid and/or LRPPRC cDNA were microinjected into 1-cell zebrafish embryos. After 24 hours post fertilization (Hpf), fluorescence microscopy was used to detect the blood vessel formation in zebrafish model. For subsequent examination of VEGFA mRNA of zebrafish embryos, qRT-PCR assay was performed following the procedure above.

Statistical analysis

Data in our study were analyzed by GraphPad Prism 8.0 and SPSS 24.0 software. Student's t test was used for statistical analyses. The Kaplan-Meier survival analysis was performed to assess OS and PFS. p value less than 0.05 was considered as statistical significance.

Abbreviations

BCA: Bicinchoninic Acid; DAPI: 4′,6-diamidino-2-phenylindole; DEGs: Differentially expressed genes; DMEM: Dulbecco's Modified Eagle's Medium; EGFP: Enhanced Green Fluorescent Protein; GO: Gene ontology; GSEA: Gene set enrichment analysis; HGSOC: High-grade serous ovarian carcinoma; Hpf: Hours post fertilization; HUVEC: Human umbilical vein endothelial cells; IB: Immunoblotting; IHC: Immunohistochemistry; IP: Immunoprecipitation; MS: Mass spectrometry; LRPPRC: Leucine-rich PPR motif-containing protein; OC: Ovarian cancer; OCT: Octamer-binding transcription factor 4; OD: Optical density; OS: Overall survival; PBS: Phosphate buffered saline; PDOs: Organoids derived from ovarian cancer patients; PFS: Progression-free survival; qRT-PCR: Quantitative real time PCR; RT: Room temperature; 3D: Three-dimensional; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; TSS: Transcription start site; VEGFR2: Vascular endothelial growth factor receptor 2.

Declarations

Authors' contributions

HZ.S and X.C conceived and coordinated the project. DL.W, WW.X and HZ.S performed the experiments. DL.W, HS.W and W.C analyzed the data and wrote the manuscript. All authors read and approved the final version of the manuscript.

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Ethics approval and consent to participate

All the animal experiments were performed following the guidelines for Institutional Animal Care and Use Committee (IACUC) and permitted by the Ethics Committee in Xinhua Hospital, Shanghai Jiaotong University School of Medical. The ovarian cancer patients’ samples were conducted under the approval of Institutional Ethics Committee (IEC) of Xinhua Hospital.

Consent for publication

All authors agreed on the manuscript.

Availability of data and materials

All data in our study are included in the article and are available from the corresponding authors.

Competing interests

The authors have declared no conflict of interest.

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Figures
Expression of OCT4 in ovarian cancer and the survival analysis. (A) Represent figures of immunofluorescence of OCT4 in ovarian cancer organoids. 8 organoids of epithelial ovarian cancer were generated in our study. (B) Immunofluorescence was applied to test the expression of OCT4 in 20 primary lesions of ovarian cancer and 20 corresponding metastatic lesions. (C) IHC analysis was performed in tissue chips containing 153 samples of benign ovarian tumor, 187 primary lesions of ovarian cancer, and...
154 metastatic specimens from greater omenta. (D-E) Statistical results of above IHC results (D: H-score; E: high expression ratio of OCT4). (F-G) OS (F) and PFS (G) analysis of ovarian cancer patients. **, P < 0.01.

Figure 2

OCT4 silencing decreases angiogenesis and cell migration in ovarian cancer. (A) mRNA expression of OCT4 tested by qRT-PCR. (B) Protein level of OCT4 detected by immunoblotting assay. β-actin was used
as the internal control. (C) Hot map of RNA-sequencing between SKOV3ip1/vector and SKOV3 silencing ovarian cancer cell groups. (D) GO-biological process analysis of DEGs between SKOV3ip1/vector and SKOV3/OCT4-sh1 ovarian cancer cells. (E) KEGG pathway analysis of DEGs between SKOV3ip1/vector and SKOV3/OCT4-sh1 ovarian cancer cells.
OCT4 participates in cell migration and invasion in ovarian cancer. (A) GSEA analysis in OCT4 silencing ovarian cancer cells and their controls (SKOV3ip1/OCT4-sh1 and SKOV3ip1/Vector). (B) The metastasis associated proteins was tested by immunoblotting assay using the indicated antibodies. β-actin was used as the loading control. (C) Wound healing assay in OCT4 silencing ovarian cancer cells and their controls. (D) Transwell assay of cell migration was used in OCT4 silencing ovarian cancer cells and their controls. (E) Transwell assay of cell invasion was used in OCT4 silencing ovarian cancer cells and their controls. (F-H) Quantification analysis of cell migration and invasion in above study (Figure 3C-F). All the experiments were conducted triplicates.
Figure 4

Angiogenesis examination and the interaction between VEGFR2 and LRPPRC. (A) GSEA analysis in OCT4 silencing ovarian cancer cells and their controls (SKOV3ip1/OCT4-sh1 and SKOV3ip1/Vector). (B-C) Tube formation affected by supernatant of OCT4 silencing ovarian cancer cell lines and the controls in HUVECs. (D) Silver staining after immunoprecipitation and SDS-PAGE assay. (E) Peptide of LRPPRC binding to VEGFR2 generated by mass spectrum analysis. (F) The interaction between LRPPRC and
VEGFR2 was verified by Co-IP. (G) Immunofluorescence assay was applied to test the association between VEGFR2 and LRPPRC in ovarian cancer cells.

Figure 5

LRPPRC is a downstream molecule of OCT4 in ovarian cancer cells. (A) LRPPRC mRNA level was tested by qRT-PCR analysis in OCT4 silencing ovarian cancer cells and the controls. (B) Immunoblotting assay of LRPPRC in OCT4 silencing ovarian cancer cells and the controls. (C) Immunofluorescence staining of...
LRPPRC in OCT4 silencing ovarian cancer cells and the controls. (D) ChIP assay suggested that OCT4 bound to specific DNA sequences of LRPPRC. (E) Immunoblot assay for determining the relationship between OCT4 and p-VEGFR2 and the downstream proteins. (F) Heat map of genes associated angiogenesis and metastasis.

**Figure 6**

A. SKOV3ip1

B. Tumor volume (mm$^3$)

C. Tumor weight (g)

D. SKOV3ip1

E. Total flux

F. Tumor weight (g)

G. OCT4, LRPPRC, DAPI, Merged

H. OCT4, CD31, DAPI, Merged

I. Vector, OCT4 morpholino, OCT4 morpholino+LRPPRC

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Figure 6
The effects of OCT4 silencing and LRPPRC overexpression on the progression of ovarian cancer and vascular formation in vivo. (A) Subcutaneous models of nude mice xenograft tumors built with SKOV3ip1/Vector, SKOV3ip1/OCT4-sh1, and SKOV3ip1/OCT4-sh1 + LRPPRC overexpression cells. (B) Growth curve of the subcutaneous models of nude mice xenograft tumors. (C) Tumor weight of the subcutaneous models of nude mice xenograft tumors. (D) Intraperitoneal models of nude mice xenograft tumors produced by living small animal imaging system. (E) The luciferin uptake value of the above imaging system. (F) Weight of intraperitoneal models of nude mice xenograft tumors. (G) Immunofluorescence of OCT4 and LRPPRC in primary ovarian cancer lesions with OCT4 silencing and the negative control in the intraperitoneal xenograft models. (H) Immunofluorescence of OCT4 and endothelial cell specific molecule CD31 in primary ovarian cancer lesions with OCT4 silencing and the negative control in the intraperitoneal xenograft models. (I) Vascular formation models of zebrafish model.
Figure 7

Association among OCT4, LRPPRC and VEGFR2 in human ovarian cancer tissues. (A) IHC assay of LRPPRC in benign ovarian tumor samples, primary lesions of ovarian cancer and the metastatic specimens gained from greater omentum. (B) Survival analysis of LRPPRC. Data was obtained from Xinhua hospital. (C) The positive ratio of LRPPRC in 153 benign ovarian tumor samples, 187 primary ovarian cancer lesions and 154 metastatic specimens form greater omentum. (D) Immunofluorescence
staining of OCT4 and CD31 in primary lesions of ovarian cancer and the metastatic tissues from greater omentum. (E) qRT-PCR assay in 30 OCT4 high expressed ovarian cancer samples and 30 OCT4 low expressed specimens. (F) OCT4/VEGFR2/LRPPRC pathway in modulating ovarian cancer cell metastasis and angiogenesis.

**Supplementary Files**

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