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OCT4 mediates FSH-induced epithelial–mesenchymal transition and invasion through the ERK1/2 signaling pathway in epithelial ovarian cancer

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ABSTRACT

Our previous study showed that Octamer-binding transcription factor 4 (OCT4) expression was upregulated and significantly associated with histological grade through the analysis of OCT4 expression in 159 ovarian cancer tissue samples, and OCT4 mediated follicle-stimulating hormone (FSH)-induced anti-apoptosis in epithelial ovarian cancer. Nevertheless, whether OCT4 participates in FSH-induced invasion in ovarian cancer is still unknown. Therefore, the present study aimed to define whether FSH-induced ovarian cancer invasion is mediated by OCT4. In present study, we showed that FSH induced not only the epithelial–mesenchymal transition (EMT) and invasive phenotype but also the upregulation of OCT4 expression in a dose- and time-dependent manner in epithelial ovarian cancer cells. In addition, the expression of FSH receptor (FSHR) was upregulated by FSH induction, and knockdown of FSHR inhibited FSH-stimulated OCT4 expression. ERK1/2 signaling pathway participated in the enhanced expression of OCT4 and Snail induced by FSH. We further showed that the activated expression of Snail and N-cadherin, the suppressed expression of E-cadherin and the morphological change of the cells stimulated by FSH were blocked by OCT4-specific small interfering RNA. Moreover, our results showed that OCT4 mediated the increase in invasive capacity induced by FSH in ovarian cancer cells. Taken together, our work reveals that OCT4 is an essential mediator in FSH-induced EMT and invasion in epithelial ovarian cancer and may act as a potential therapeutic target.

1. Introduction

Ovarian carcinoma is one of the most common reproductive system malignant cancers, with an overall mortality rate of 50% in developed countries [1]. Over 90 percent of ovarian cancer cases are epithelial ovarian cancer (EOC) [2]. The high mortality of ovarian cancer is mainly because 60% of the patients are diagnosed with having advanced disease with widespread intraperitoneal metastasis and ascites [3]. Therefore, new therapeutic targets to treat the invasion and dissemination of ovarian cancer are needed. However, the molecular mechanisms underlying ovarian cancer metastasis are poorly understood.

Ovarian cancer is more common among patients with rising gonadotropins, including follicle-stimulating hormone (FSH) and luteinizing hormone (LH), for example, postmenopausal women or patients going through ovulation induction [4,5]. In addition, a lower level of gonadotropins exposure, such as a high number of pregnancies, contraceptive use and breast-feeding, has been positively correlated with a decreased incidence of ovarian cancer [6,7]. Moreover, the levels of gonadotropins appear to be elevated among ovarian carcinoma and primary peritoneal cancer patients. Taken together, this evidence indicates that gonadotropins may have a key role in ovarian carcinoma development and progression. This hypothesis was supported by the results of recent studies that have demonstrated that the treatment of ovarian cancer cells with gonadotropins led to an increased metastatic and invasive ability [8,9]. Gonadotropins also induced the expression of certain genes that were known to participate in angiogenesis or metastasis, including OCT4.

OCT4 is an important transcription factor that participates in regulating self-renewal and pluripotency in undifferentiated embryonic stem cells [10]. Abnormal OCT4 expression has been observed in many cancers. Previously, we demonstrated that OCT4 expression was significantly associated with histological grade through the analysis of the OCT4 expression in 159 patients ovarian

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tissue samples, and OCT4 mediated FSH-induced anti-apoptosis in epithelial ovarian cancer [11]. However, the detailed mechanisms underlying the role of OCT4 in FSH-induced cell invasion in epithelial ovarian cancer still remain to be elucidated.

EMT is a process that is necessary for metastasis and invasion of epithelial cancers. Transcription factors, including Slug and Snail, participated in the EMT process. EMT is characterized by a change in cell morphological specificity due to the loss of epithelial markers, such as E-cadherin, and the acquisition of mesenchymal characteristics, such as N-cadherin. This is accompanied by an increase in cell motility and invasiveness. In the present report, our results showed the presumptive mechanism by which OCT4 mediated FSH-induced EMT and invasive phenotype in epithelial ovarian carcinoma.

2. Materials and methods

2.1. Cell cultures

American Type Culture Collection provided the ovarian cancer cell lines, Hey and HO8910. These cells were maintained in an DMEM/F12 medium supplemented with 10% fetal bovine serum at37 °C under 5% CO_2.

2.2. siRNA transfection

Human FSHR siRNA and Control siRNA were synthesized by GenePharma (Shanghai, China). The siRNA targeting OCT4 and Control siRNA were obtained from Santa Cruz, USA. According to the manufacturer's instructions, and the sequences of siRNA corresponding to the OCT4 gene included: 5′-GCGAUCAAGCAGCGACUAU-3′,5′-UCCCAUGCAUUCAAACUGA-3′,5′-GCACUGUACUCCUCGGUCC-3′,5′-CGAGAAGGAUGUGGUCCGA-3'. These siRNA were transiently transfected into the cells with Lipofectamine 2000 (Invitrogen, USA). After incubation for another 24 h, a portion of the cells was collected for RNA extraction and qRT-PCR analysis to determine the degree of gene silencing and detected the effect of knocking down OCT4. The other portion of the treated cells was used to investigate the effect of OCT4 depletion on downstream proteins by western blotting analysis in the absence or presence of FSH.

2.3. Western blotting

Western blotting were performed as previously described [12]. In brief, the proteins of cells stimulated with FSH or inhibitors were extracted, and the proteins were separated and transferred to PVDF membranes. The corresponding specific antibodies were used for proteins of interest identification. The antibodies against N-cadherin, E-cadherin and Snail were obtained from Cell Signaling Technology (Beverly, MA). The antibodies against FSHR and OCT4 were obtained from Abcam (Cambridge, UK). The antibodies against GAPDH was obtained from Epitomics (CA, USA).

2.4. RNA extraction and qRT-PCR

According to the manufacturer's instructions, TRIzol (Invitrogen) was used to extract total RNA. Reverse transcription was carried out as previously described [11]. The primers for FSHR, OCT4, Snail, E-cadherin, N-cadherin and GAPDH genes were obtained from Invitrogen Bioengineering Corporation (Shanghai, China). qRT-PCR was performed with 2 μl cDNA template, 1 μl primer mixture and 10 μl SYBR Green PCR Master Mix. The determination of mRNA levels is represented with an average value, and the qRT-PCR experiments were repeated at least three times.

2.5. Transwell invasion assays

Hey and Ho8910 (1×10^5) cells transfected with si-Con or si-OCT4 were seeded in the upper chamber of 24-well transwell plates (Corning, NY, USA) coated with BD matrigel basement membrane matrix with serum-free medium. DMEM/F12 supplemented with 10% fetal bovine serum was added to the lower chamber. After incubating for 16 h, the invasive cells were counted under a microscope. All the samples were plated in triplicate.

2.6. Statistical analysis

All the data are presented as the mean ± standard deviation. Student's t test was used to analyze the difference between groups. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS 20.0.

3. Results

3.1. FSH induces the EMT and invasive phenotype in epithelial ovarian cancer cells

EMT plays an important role in cancer progression. To identify whether FSH was involved in ovarian cancer EMT and invasion, different concentrations of FSH were used to stimulate the ovarian cancer cell lines, Hey and Ho8910. We found that the protein and mRNA levels of the epithelial marker, E-cadherin, were dramatically downregulated after FSH treatment in a dose-dependent manner ($P < 0.05$) (Fig. 1A and B). On the contrary, the expression of the mesenchymal marker, N-cadherin, was significantly upregulated in a dose-dependent manner ($P < 0.05$) (Fig. 1A and B). Consistent with these findings, a morphological transition from a round-like shape to a spindle-like appearance was observed among Hey and Ho8910 cells stimulated with FSH (Con and 50 mIU/ml) (Fig. 1C). In addition, significantly increased invasion was demonstrated in ovarian cancer cells induced by FSH (Con and 50 mIU/ml), as determined by the transwell invasion assays ($P < 0.05$) (Fig. 1D). In summary, these data show that FSH induces EMT and invasion in epithelial ovarian cancer.

3.2. FSH treatment upregulates the expression of FSHR and OCT4, and FSHR is required for the FSH-stimulated the expression of OCT4

Because FSH played a crucial role in ovarian cancer EMT and invasion, we hypothesized that certain genes activated by FSH were involved in EMT and progression of ovarian cancer. In support for our hypothesis, we showed that the protein expression level of OCT4 were significantly upregulated in dose- (Fig. 2A) and time-dependent manners after FSH stimulation (Fig. 2B). Many studies have shown that alterations in the FSHR participated in the regulation of ovarian responsiveness to FSH [13–16]. We therefore examined whether the effect of FSH on ovarian cancer metastasis was correlated with the level of FSHR expression. As shown Fig. 2C and D, FSH treatment resulted in a dose-dependent upregulation of FSHR mRNA and protein expression in Hey and Ho8910 cells ($P < 0.05$). These data demonstrate that FSH treatment upregulates the expression of FSHR and OCT4.

To further demonstrate the role of FSHR in the expression of OCT4, we knocked down FSHR using siRNA. As shown in Fig. 2E and F, the effectiveness of silencing FSHR by siRNA was verified through western blotting, and the results were consistent with a previous study *($P < 0.05$)* [17,18]. Importantly, siRNA mediated depletion of FSHR could block the effects of FSH on OCT4 protein and mRNA expression levels ($P < 0.05$) (Fig. 2E and F). These results suggest that FSHR is indispensable for FSH-induced OCT4 expression.

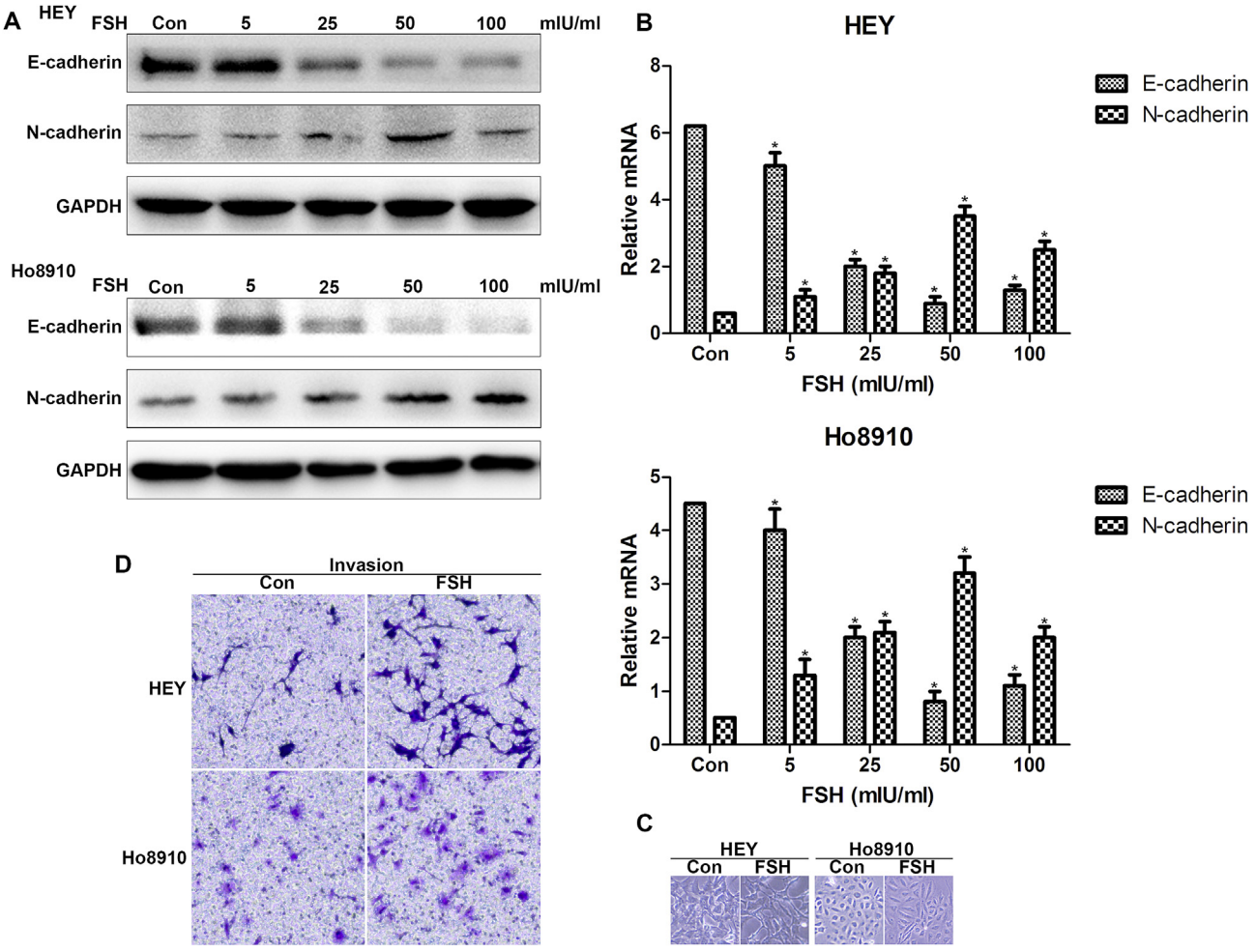

Fig. 1. FSH induces the EMT and invasive phenotype in ovarian cancer cells. (A) and (B) qRT-PCR and western blotting were used to assess the mRNA and protein expression of E-cadherin and N-cadherin in the ovarian cancer cells Hey and Ho8910 stimulated with increasing doses of FSH. (C) Hey and Ho8910 cells were stimulated with FSH (Con and 50 mIU/ml), and cells morphology was assessed microscopically. (D) The invasive ability of Hey and Ho8910 cells treated with FSH (Con and 50 mIU/ml) was examined by transwell assays. *$P < 0.05$ compared with Con.

3.3. ERK1/2 is involved in the upregulation of OCT4 and Snail expression induced by FSH

ERK1/2 signaling pathway was reported to participate in the EMT of ovarian cancer [19]. Nevertheless, the molecular mechanisms involved in FSH-induced EMT remain to be completely elucidated. To investigate the molecular signaling underlying FSH-stimulated the expression of OCT4, signaling pathway inhibitors against PI3K/Akt (LY294002), ERK1/2 (U0126) and ROS (NAC) were used to treat the ovarian cancer cell lines, Hey and Ho8910. Western blotting showed that the enhanced expression of OCT4 induced by FSH was attenuated when Ho8910 and Hey cells were pretreated with ERK1/2 (U0126)-specific inhibitors ($P < 0.05$), but not when pretreated with PI3K/Akt (LY294002)- or ROS (NAC)-specific inhibitors (Fig. 3A). These results suggest that ERK1/2 signaling pathway participates in FSH-induced the expression of OCT4. EMT transcription factor, Snail, is of vital importance in the metastasis of ovarian carcinoma, we explored whether ERK1/2 signaling pathway participated in the upregulation of Snail expression induced by FSH. qRT-PCR and western blotting results showed that Hey and Ho8910 cells treated with U0126 blocked the FSH-induced the upregulation of Snail ($P < 0.05$), while the ROS and PI3K/Akt signaling pathways inhibitors had no effect on Snail expression (Fig. 3B and C). These data demonstrate that ERK1/2 signaling pathway participates in the expression of OCT4 and Snail induced by FSH.

3.4. OCT4 mediates FSH-induced the EMT and invasion in ovarian cancer cells

To confirm whether OCT4 taked part in the expression of Snail induced by FSH, OCT4 expression was silenced in Hey and Ho8910 transfected with si-OCT4. Western blotting and qRT-PCR demonstrated that si-OCT4 transfection significantly diminished the upregulation of the protein and mRNA levels of Snail expression induced by FSH ($P < 0.05$) (Fig. 4A and Fig. S1). Taken together, these data demonstrate that OCT4 is indispensable for FSH-stimulated Snail expression in ovarian cancer. To determine whether the activation of N-cadherin expression and the suppression of E-cadherin expression induced by FSH were mediated by OCT4. FSH induced a morphological change in Hey and Ho8910 cells, whereas the morphology of OCT4-depleted cells remained unchanged (Fig. 4B). Similarly, OCT4 siRNA treatment attenuated the downregulation of E-cadherin and upregulation of N-cadherin induced by FSH ($P < 0.05$) (Fig. 4C and Fig. S2). In summary, these results suggest that OCT4 is necessary for N-cadherin and E-cadherin expression during the FSH-induced EMT process. To examine whether OCT4 was involved in the migration and invasion of

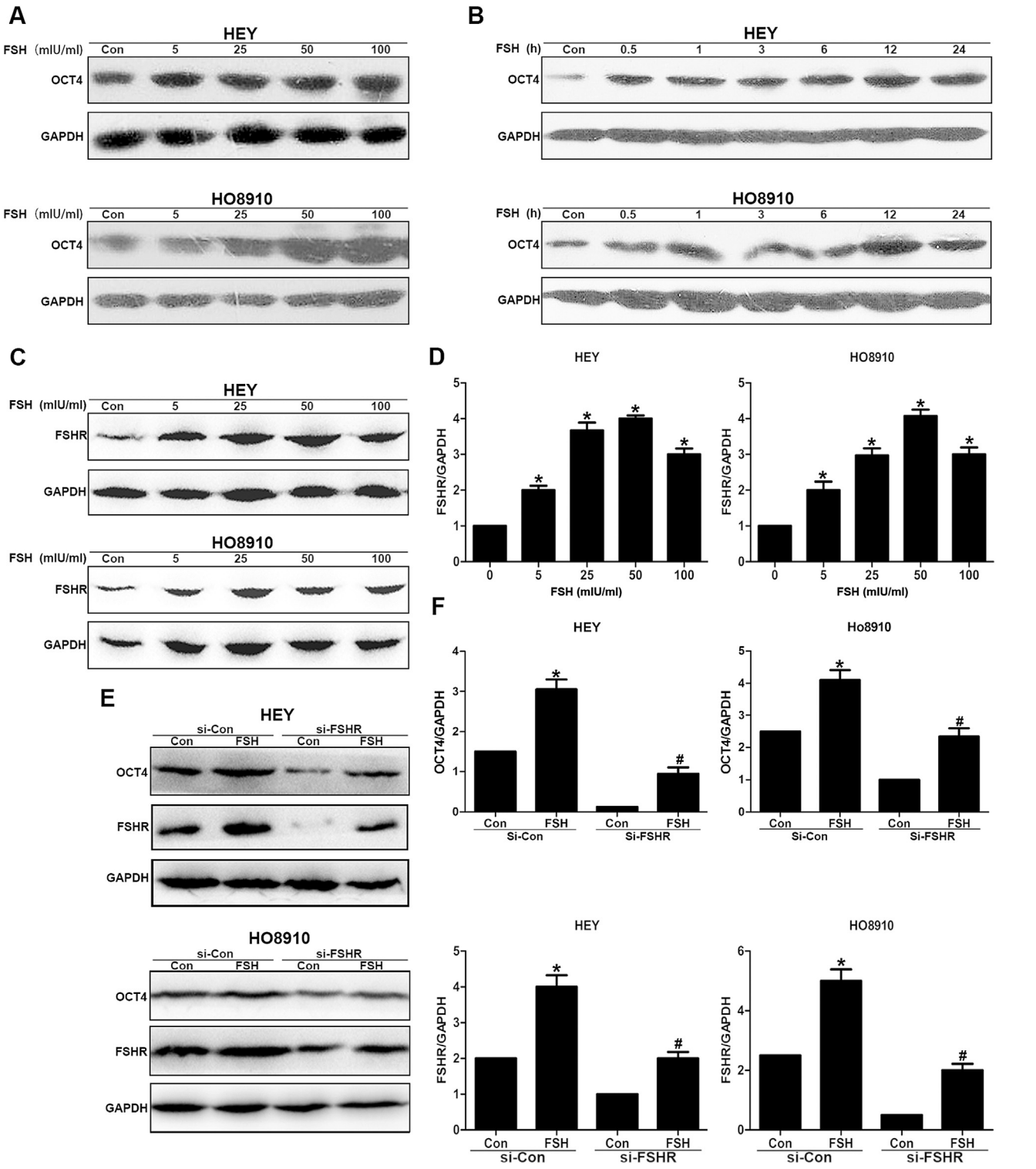

Fig. 2. FSH treatment upregulates the expression of FSHR and OCT4, and FSHR is required for FSH-stimulated OCT4 expression in epithelial ovarian cancer cells. (A) Different concentrations of FSH were used to stimulate Hey and Ho8910 cells, and western blotting was used to analyze the expression of OCT4. (B) Hey and Ho8910 cells were stimulated with 50 mIU/ml FSH at different time points, and western blotting was used to examine OCT4 expression. (C) and (D) qRT-PCR and western blotting were used to analyze FSHR mRNA and protein levels in Hey and Ho8910 cells stimulated with 50 mIU/ml FSH at different time points. (E) and (F). qRT-PCR and western blotting were used to examine the expression of FSHR and OCT4 in Hey and Ho8910 cells transfected with si-Con or si-FSHR and stimulated with 50 mIU/ml FSH. $^{\#}P < 0.05$ compared with FSH stimulation in si-Con group. $^{*}P < 0.05$ compared with Con in si-Con group.

epithelial ovarian cancer cells induced by FSH, the migratory and invasive capacity of si-Con or si-OCT4 transfected Hey and Ho8910 cells stimulated with FSH were estimated through wound healing assays and transwell invasion assays. FSH-induced invasive and migratory capacity was reduced in cells transfected with si-OCT4 ($P < 0.05$) (Fig. 4D and Fig. S3). In conclusion, our data show that OCT4 is a mediator of the EMT and invasion induced by FSH in epithelial ovarian cancer.

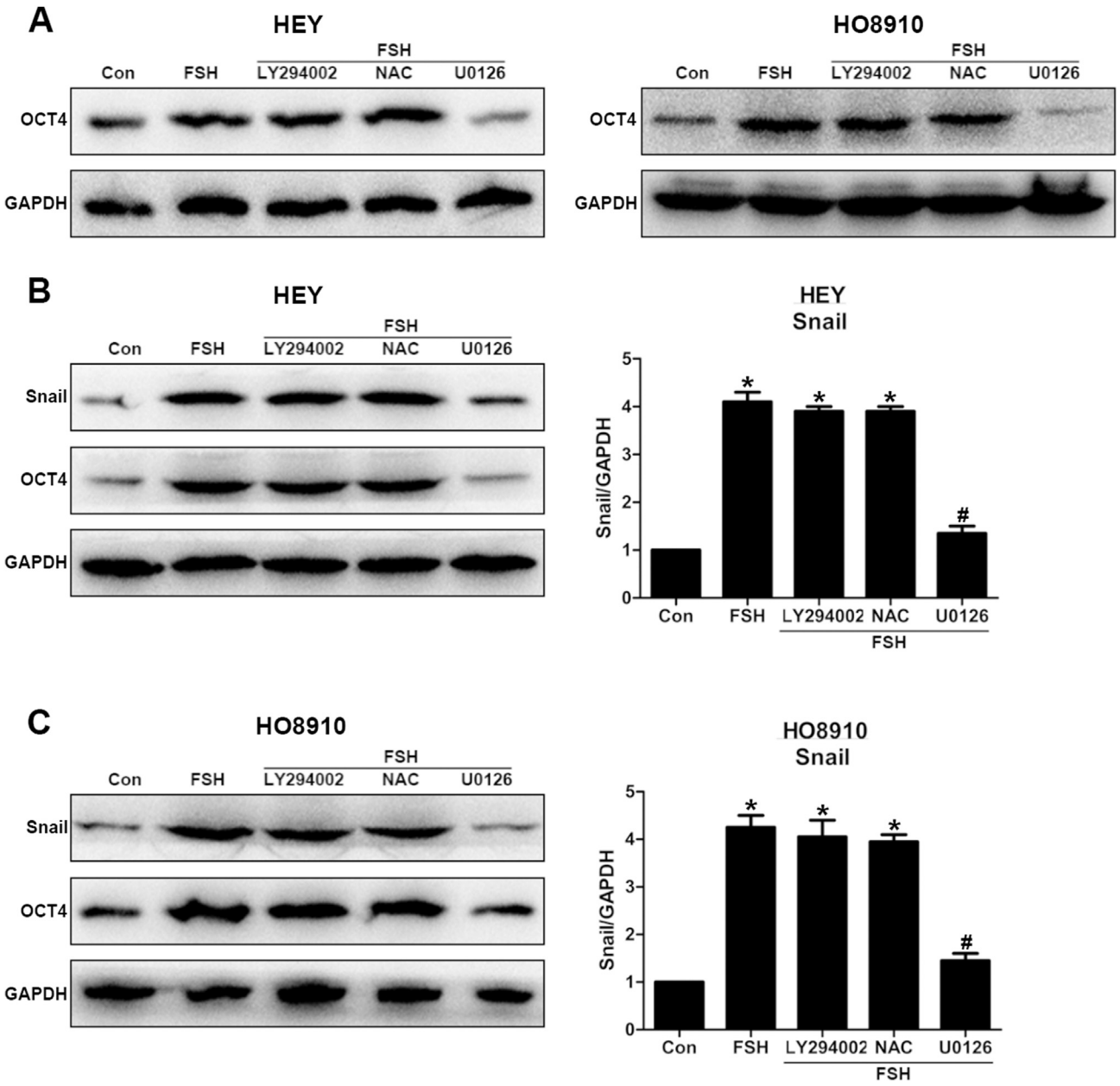

Fig. 3. ERK1/2 participates in the induction of OCT4 and Snail expression by FSH. (A) Hey and Ho8910 cells were pretreated with inhibitors against PI3K/Akt (LY294002), ERK1/2 (U0126) and ROS (NAC), and then stimulated with 50 mIU/ml FSH. Western blotting was used to examine the protein level of OCT4. (B) and (C) qRT-PCR and western blotting were used to assess the mRNA and protein levels of Snail. $^{\#}P < 0.05$ compared with the FSH treatment. $^{*}P < 0.05$ compared with the Con.

4. Discussion

The previous study showed that OCT4 mediated FSH-induced anti-apoptosis in epithelial ovarian cancer cells [11]. However, whether OCT4 participates in the effects of FSH on epithelial ovarian cancer EMT and invasion are still unclear. Therefore, the present work was aimed at investigating the relationship between FSH, OCT4, EMT and invasion in epithelial ovarian cancer. We demonstrated that FSH induced epithelial ovarian cancer invasion via the regulation of ERK1/2 signaling pathway, resulting in the upregulation of OCT4 expression and subsequently EMT and invasion.

Gonadotropins have been found in the ovarian tumor fluid of the majority of ovarian cancer patients, indicating that gonadotropins have crucial role in the transformation and progression of ovarian cancer [20–23]. *Moreover, FSH may be the main hormone associated with ovarian cancer, and a higher level of FSH in EOC was correlated with a poor prognosis* [16]. Because the acquisition of EMT phenotype is a key step for cancer metastasis, we sought to determine whether FSH induced EMT and ovarian cancer progression. EMT was induced by a sophisticated interplay between tumor cells and their surrounding environment, including growth factors and cytokines [19]. Previous studies have reported that TGF-β, norepinephrine (NE), estrogen and EGF induced EMT and ovarian cancer aggressiveness [24–27]. *It was also reported that FSH induced EMT and promoted metastasis in ovarian cancer cells* [18]. Consistent with the previous studies, our results manifested that FSH induced the upregulation of N-cadherin expression and the downregulation of E-cadherin expression in a dose-dependent manner. Moreover, a morphological transition from a round-like shape to spindle-like

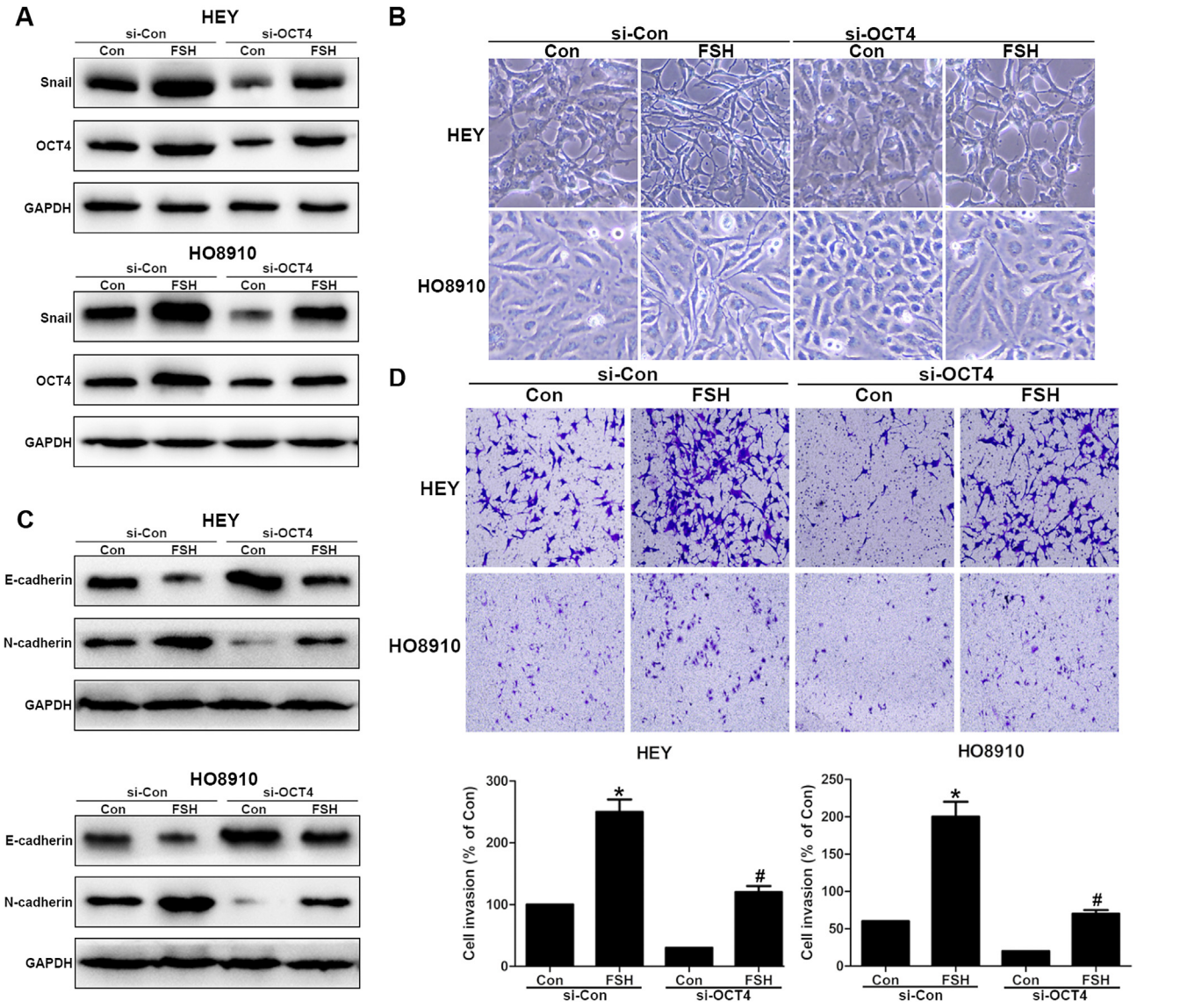

Fig. 4. OCT4 mediates FSH-induced the EMT and invasion in ovarian cancer cells. FSH (50 mIU/ml) or Con were used to stimulate Hey and Ho8910 cells transfected with si-Con or si-OCT4. (A) Western blotting was used to examine the protein expression of OCT4 and Snail. (B) The morphology of the cells was assessed microscopically. (C) Western blotting were used to analyze the protein levels of E-cadherin and N-cadherin. (D) Cells invasion was examined by transwell invasion assays. $^{\#}P < 0.05$ compared with the FSH treatment in si-Con group. $^{*}P < 0.05$ compared with the Con in si-Con group.

appearance and enhanced cells invasive ability were also observed in ovarian cancer cells stimulated with FSH. Therefore, these data indicate that FSH induces EMT and ovarian cancer invasion.

Many studies have investigated the role of gonadotropins on ovarian cancer, and have shown that gonadotropins facilitated ovarian cancer proliferation and metastasis through the regulation of key genes [28]. For instance, proteinase expression was upregulated by gonadotropins in ovarian cancer cells, which resulted in gonadotropin-dependent changes in invasive and migratory capability [8]. Recently, it was reported that gonadotropins promoted the migration and invasion of ovarian cancer cells by increasing cyclooxygenases expression and the production of prostaglandin E(2) [29]. Moreover, it was showed that FSH stimulation led to increased number of stem cells with increased expression of Nanog and *OCT4A in ovarian surface epithelium cells (OSE)* [16]. FSHR play an important role in the regulation of ovarian responsiveness to FSH. *FSH-FSHR3-stem cell network in OSE may probably be involved in various pathologies such as cancer* [16]. *Meanwhile, it was reported that FSH induced EMT and invasion through FSHR in ovarian cancer* [18]. In agreement with previous study, the current study shows that FSH treatment upregulates the expression of FSHR and OCT4, and FSHR is required for the FSH-stimulated the expression of OCT4.

MAPKs have a key role in transmitting signals from external environment stimulation, including chemotherapeutics, stress and hormones, to cellular responses, such as apoptosis, proliferation and differentiation. ERK is one of the MAPKs family members [30], and both the receptor tyrosine kinases, EGFR and GPRs, can activate MAPK signaling pathways [31,32]. FSH and LH produced biological effects through their corresponding GPRs. A recent study reported that gonadotropins activated the MAPKs signaling pathway in immortalized ovarian surface epithelial cells, leading to the upregulation of EGFR [33,34]. Gonadotropins induced proliferation and migration through the regulation of ERK1/2 signaling pathway in ovarian cancer cells [35]. These studies suggest that MAPKs have a key role in the biological effects induced by gonadotropins. In our

present study, FSH stimulated ovarian cancer cells pretreated with ERK1/2 inhibitor, failed to upregulate OCT4 and Snail expression. These results suggest that ERK1/2 takes part in the expression of OCT4 and Snail induced by FSH in ovarian cancer.

OCT4 participated in regulating pluripotency and self-renewal in primordial germ and embryonic stem cells. It was recently reported that OCT4 played multi-functional roles in cancer development. Metastasis is a major obstacle to treating for ovarian cancer. The specific molecular changes in EOC cells that promote the metastatic process are largely undefined. OCT4 was previously reported to promote cancer metastasis through EMT [36–38]. Increasing evidence demonstrated that cancer cells metastasis was closely related with the EMT process. Our previous study showed that upregulated OCT4 expression contributed to FSH-induced apoptosis inhibition in ovarian cancer [11]. These prompted us to determine whether OCT4 was involved in EMT and invasion induced by FSH in ovarian cancer.

EMT is necessary for the acquisition of migratory and invasive ability of epithelial cells. A previous study showed that the expression E-cadherin was negatively correlated with invasion and positively associated with prognosis in ovarian cancer [39]. Thus, the invasiveness of ovarian cancer was possibly suppressed by E-cadherin and the loss of the expression of E-cadherin was reported to contribute to metastasis in ovarian cancer [40]. In the present study, we found that OCT4 was indispensable for the FSH-induced the activation of Snail and N-cadherin and the suppression of E-cadherin, subsequently resulting in EMT and invasion in ovarian cancer cells.

Taken together, the present study demonstrated that FSH stimulation increased the expression of OCT4 via the activation of FSHR and ERK1/2 signaling pathway. OCT4 upregulated Snail expression to downregulate E-cadherin and upregulate N-cadherin, subsequently contributing to FSH-induced EMT and cell invasion in epithelial ovarian cancer.

Conflict of interest

The authors declare that there is no conflict of interests.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.04.061.

Transparency document

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