

Expression of microRNA-145, OCT4, and SOX2 in double primary endometrioid endometrial and ovarian carcinomas

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Summary. Double primary endometrioid endometrial and ovarian carcinomas (DPEEOCs) are the most common multiple gynecological carcinomas. In recent years, gene sequential comparison analysis has strongly supported the opinion that sporadic double endometrioid endometrial and ovarian cancers (DEEOCs) are clonally related in both primary and metastatic tumors. In order to find more clonal evidence for DPEEOC, we investigated cancer stem cells (CSCs). SOX2 and OCT4 are two common factors in CSCs. MicroRNA (miRNA)-145, a small non-coding RNA, has effects in regulating gene expression and tumorigenesis in CSCs. The aim of this study was to assess the involvements of SOX2, OCT4, and miRNA-145 in the tumorigenesis of DPEEOCs. In our study, twenty DPEEOC patients were chosen. Metastatic DEEOCs and normal endometrial and ovarian tissues were also included. The expression of miRNA-145 was detected by real-time quantitative PCR. Immunohistochemical staining was used to measure the expression of OCT4 and SOX2. The results showed that miRNA-145 expression was lower in DPEEOC endometrial tissues and higher in DPEEOC ovarian tissues compared to the corresponding normal tissues. Both SOX2 and OCT4 were over-expressed in cancer tissues compared with that in normal tissues. MiRNA-145, SOX2, and OCT4 were expressed at similar levels in two cancer sites of a given DPEEOC or metastatic DEEOC sample. Besides, metastatic DEEOC

sections expressed a higher level of SOX2 and OCT4 compared to the corresponding DPEEOC tissues. Together, these results support the clonality of DPEEOCs. Moreover, SOX2 and OCT4 may have some implication in DPEEOC and metastatic DEEOC diagnosis.

Key words: Endometrial adenocarcinoma, Ovarian carcinoma, OCT4, SOX2, MicroRNA-145

Introduction

Double endometrioid endometrial and ovarian carcinomas (DEEOCs) are the most common gynecological synchronous neoplasms, occurring in almost 4.2% of patients with uterine carcinomas and 10% of patients with ovarian cancer (Zaino et al., 2001). Both sections of the tumors may contain neoplastic glands that appear similar to normal endometrial glands under a microscope. DEEOCs often present in younger patients, and most patients have lower grade cancers and a better prognosis. Most DEEOCs are diagnosed as double primary endometrioid endometrial and ovarian carcinomas (DPEEOCs) (Schultheis et al., 2016). DPEEOCs are primarily diagnosed according to the clinical and pathological criteria outlined by Ulbright and Roth (1985). The clinical and pathological guidelines of Scully et al. (1998) are also used as supplements to confirm the primary involvement of both cancers. In recent decades, many studies have been performed on double endometrial and ovarian cancers (DEEOCs), including investigations on microsatellite

instability, loss of heterogeneity, gene mutations (*CTNBL1*, *PTEN*, *K-RAS*, and others), and inactivation of the X chromosome (Shenson et al., 1995; Emmert-Buck et al., 1997; Lin et al., 1998). Most of these studies focused on specific factors or genes. However, the pathogenesis and etiology of DPEEOCs have not yet been elucidated. Moreover, no uniform results have been obtained regarding the clonality of DEOCs. Several researchers have studied DEEOC using gene sequencing. Chao et al. (2016) performed a genomic characterization of double cancers in DEOC and discovered that thirteen of fourteen pairs of DEEOC tumors shared somatic mutations, which is indicative of clonality. Schultheis et al. (2016) studied the clonality of DEEOCs through massively parallel sequencing. They analyzed the extent of clonality based on sequential comparison analysis and demonstrated that all sporadic DEEOCs were clonally related within a given cancer patient.

Compared to normal cells, tumor cells are poorly differentiated and proliferate indefinitely. They also have the characteristics of self-renewal and pluripotency, similar to those found in embryonic stem cells (ESCs). Both endometrial and ovarian carcinomas were proven to display cancer stem cell (CSC)-like features (Mummery et al., 2011; Wang et al., 2017). Past studies have supported the hypothesis that CSCs originate from the dedifferentiation of somatic cells. It is well known that during embryonic development, both the uterus and the ovary are derived from the secondary Müllerian system. Similarly, cancer cells in DPEEOC might have some correlation with CSCs dedifferentiating from somatic cells. Same embryonic origin and similar characteristics have led researchers to hypothesize that similar cancers share a similar origin. We designed this study to find more evidence for clonality at the CSCs angle. In CSCs, SRY-box 2 (SOX2) and octamer-binding transcription factor 4 (OCT4) are two commonly found factors. Major changes in their expression have previously been found in single endometrioid endometrial carcinomas (EEC) and endometrioid ovarian cancers (EOC) (Xu et al., 2009; Wu et al., 2011).

SOX2 (a member of the SRY-related family of proteins) is a stem cell transcription factor (Chen et al., 2014) and a well-known ESC marker. It plays an important role in ESC differentiation as well as tumorigenesis (Mu et al., 2017). Studies have demonstrated that SOX2 is highly expressed in many different cancer types, including oral squamous cell carcinomas, breast carcinomas, and colorectal tumors. It has often shown a strong correlation with a poor prognosis in many different cancers (Lundberg et al., 2016; Ren et al., 2016b; Liu et al., 2017). Li et al. (2017) discovered that SOX2 can accelerate the progress of LSCC by targeting cyclin D1. Herreros-Villanueva et al. (2013) reported that SOX2 was an important stem cell-like marker in pancreatic cancer cells; it played an important role in dedifferentiation and tumorigenesis in pancreatic cancer. Rudin and his collaborators found that

SOX2 was frequently amplified in small cell lung cancer (Rudin et al., 2012). OCT4 (a member of the POU family of proteins) is another well-known stem cell transcription factor. Similar to SOX2, it is a stem cell marker (Niwa et al., 2000). Many researchers have demonstrated that the over-expression of OCT4 might promote embryonic cell regeneration and maintain the pluripotency (Tai et al., 2005). Moreover, OCT4 was also found to be potentially beneficial for somatic cell dedifferentiation (Gidekel et al., 2003). Additionally, studies have shown that the over-expression of OCT4 is correlated with many cancer types, such as oral squamous cell carcinoma and lung cancer (Nakatsugawa et al., 2011; Bayo et al., 2015). Abnormal expression of OCT4 can participate in tumorigenesis and tumor development (Li et al., 2017). Hu et al. (2011) discovered that OCT4 could lead to epithelial mesenchymal transition by increasing Ca²⁺ influx in breast cancer. Another report demonstrated that increased expression of OCT4 in tumors led to a substantially worse survival rate in patients (Chiou et al., 2010).

Both SOX2 and OCT4 can be mediated by many genes found in CSCs and ESCs. MicroRNA (miRNA)-145 (a small non-coding RNA) has been proven as a mediator of their expression in ESCs and many CSCs (Xu et al., 2009; Zhou et al., 2017). The aim of this study was to determine the involvement of miRNA-145, OCT4, and SOX2 in DPEEOC formation. In this study, twenty pairs of DPEEOC samples were analyzed. In addition, ten normal endometrial tissues (NET) and ten normal ovarian tissues (NOT) were included. In order to enhance the robustness of our results, ten metastatic DEEOC (MDEEOC) samples were also included. Small RNAs were isolated from all formalin-fixed paraffin-embedded (FFPE) tissues containing more than 50% cancer cells, followed by reverse transcriptase quantitative PCR (RT-qPCR). The expression of OCT4 and SOX2 were measured by immunohistochemistry (IHC) staining. Samples with a positive staining were quantitatively analyzed using Image-Pro Plus.

Materials and methods

Patients

Twenty DPEEOC patients were enrolled in this study (Table 1). Ten cases of DMDEEOC were also included. The mean age of the DPEEOC patients was 49.5 years (range: 37 to 59 years). Additionally, we carefully reviewed the medical records of all patients. Tumors combined with other pathological types (e.g., serous, mucinous, and clear cell tumors) or tumors that could not be confirmed as primary or metastatic cancers were eliminated. The above cases were enrolled from the Pathology department of the Affiliated Hospital of Qingdao University, China. In addition to the DEEOC patients, ten samples from NETs and ten samples from NOTs were obtained. FFPE sections were assessed by

Tumorigenesis in DPEEOC

two doctors with experience in pathological analysis to confirm the diagnosis of DPEEOC. Both doctors were given minimal information about the patients to prevent bias. The criteria for diagnosis of DPEEOC and DMEEOC were those outlined by Ulbright et al. (Ulbright and Roth, 1985) and Scully et al. (Scully et al., 1998). The tumor stage was diagnosed according to the guidelines of the International Federation of Gynecology and Obstetrics (FIGO). Our study was approved by the local ethical committee and informed consent was obtained from all participants.

Isolation of small RNAs

All FFPE examples were saturated in 10% neutral-buffered formalin before being embedded in paraffin. Only FFPE tissues containing over 50% cancer cells were chosen. Next, these FFPE blocks were cut into 10- μ m slides. Four slides were put into RNase-free tubes. Total RNA, mainly small RNA, was isolated from FFPE tissues according to the manufacturer's guidelines using the miRNeasy FFPE Mini Kit (Tiangen, Beijing, China, no. DP502). Then, a spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA) was used to measure the concentration and purity of the RNA. All RNA was confirmed to have a purity of 1.8-2.0 absorbance ratio at 260 and 280 nm. A final volume of 40 μ l of RNA was obtained.

RT-qPCR

Reverse transcription to cDNA was performed using

4 μ l of RNA with the miRNA first-strand cDNA synthesis Kit (no. PC4801; Alabid, China) on an iCycler iQ system (BioRad Laboratories, USA) according to the manufacturer's protocol. First, poly(A) tails were added at the 3'-UTR of the miRNA (4 μ l). The reaction was then performed for 50 min at 42°C, followed by 70°C for 15 min to inactivate the TUREscript H-RTase.

PCR was performed at a total volume of 20 μ l, consisting of 1 μ l of cDNA, 8.2 μ l of ddH₂O, 10 μ l of SYBR Green Mix (Alabid), and 0.4 μ l of each RNA primer. PCR reactions for each sample were performed in duplicate on a thermal cycler (Funglyn Biotech, China) according to the manufacturer's instructions. The reaction was started with a polymerase activation step for 10 s at 95°C, followed by 40 cycles of denaturation at 94°C for 90 s and annealing/extension at 60°C for 60 s. SnRNAU6 was chosen for normalization of the data because it was stably expressed in endometrial and ovarian tissues. Forward primers for miRNA-145 (5'-TGTCAGTTTTCCCAGGAATCCCT-3') and U6 (5'-CGCTATCGCTTCGGCAGCACA-3') were designed and synthesized by Shanghai Biological Engineering Co., Ltd. (China); reverse primers targeted on poly(A) tails were synthesized by Alabid. Relative miRNA-145 expression in each FFPE tissue was calculated using the relative comparative C_t method ($2^{-\Delta\Delta C_t}$).

Immunohistochemical staining

FFPE tissues were cut into 4- μ m sections for IHC analysis. The samples were exposed to 3% H₂O₂ for 10 min to inhibit endogenous peroxidases. FFPE tissues

Table 1. Clinical pathological features of twenty DPEEOC patients.

Patient Number	Age (year)	Pathological type of both uterus and ovarian		Clinical and Pathological Stage of Endometrial Cancer		Clinical and Pathological Stage of Ovarian Cancer	
				Stage	Grade	Stage	Grade
1	54	E	E	IA	G2	IA	G2
2	41	E	E	IA	G2	IA	G2
3	56	E	E	IA	G2	IA	G2
4	56	E	E	IA	G2	IA	G2
5	37	E	E	IA	G2	IA	G2
6	52	E	E	IA	G1	IA	G3
7	44	E	E	IB	G2	IA	G2
8	57	E	E	IA	G1	IA	G1
9	50	E	E	IA	G2	IA	G2
10	59	E	E	IA	G2	IA	G2
11	53	E	E	IA	G1	IA	G2
12	49	E	E	IA	G2	IB	G2
13	53	E	E	IA	G2	IA	G2
14	45	E	E	IA	G2	IA	G3
15	47	E	E	IA	G2	IA	G2
16	52	E	E	IB	G2	IA	G2
17	42	E	E	IA	G1	IA	G2
18	46	E	E	IA	G2	IA	G2
19	47	E	E	IA	G2	IA	G2
20	50	E	E	IA	G2	IB	G2

E refers to endometrioid type under the microscope; G refers to differentiated grade; stage follows FIGO stage.

were then immersed in sodium citrate buffer (pH=6.0) or EDTA buffer (pH=8.0) for antigen retrieval. The solution was incubated in an autoclave for 3 min at 100°C. Rabbit anti-human OCT4 monoclonal antibody (1:15 dilution; ENT3233, Elabscience, China) and polyclonal antibodies against SOX2 protein (1:10 dilution; ESAP10756, Elabscience) were used. The primary antibodies were then incubated at 4°C overnight in a wet box. Then polymer-HRP (Zhongshan Jinqiao) was added, and the tissues were incubated at 37°C for 30 min. Finally, the tissues were visualized with DAB (Zhongshan Jinqiao) and counterstained with hematoxylin. Negative-control slides were incubated with PBS instead of primary antibody.

The results of IHC were categorized based on the following criteria-0: <5% positive cells, 1: ≥5% positive cells. Stain intensity-0: no staining; 1: faint yellow; 2: yellow-brown or dark-brown. The expression level (the product of the two scores) was finally defined as negative (0) or positive (1-2). The positive samples were quantified using Image-Pro Plus (Media Cybernetics, USA). Three pictures were randomly chosen from every positive sample. Information regarding the staining

intensity was recorded.

Statistical analysis

SPSS 19.0 (IBM, USA) was used for all statistical calculations. When comparing between endometrial and ovarian tumors in DEEOC patients, the paired Wilcoxon non-parametric test or Student's t test was used for statistical analysis. The Wilcoxon signed-rank test or Student's t test was used to compare unpaired groups. The correlation between miRNA-145, OCT4, and SOX2 expression was calculated using Pearson's correlation coefficient. Two-tailed tests were utilized for all the above tests. A value of $P < 0.05$ was considered statistically significant.

Results

IHC analysis

IHC staining was performed on all FFPE tissues. Both SOX2 and OCT4 were found to be expressed in EEC (Fig. 1) and EOC tissues (Fig. 2). SOX2 and OCT4

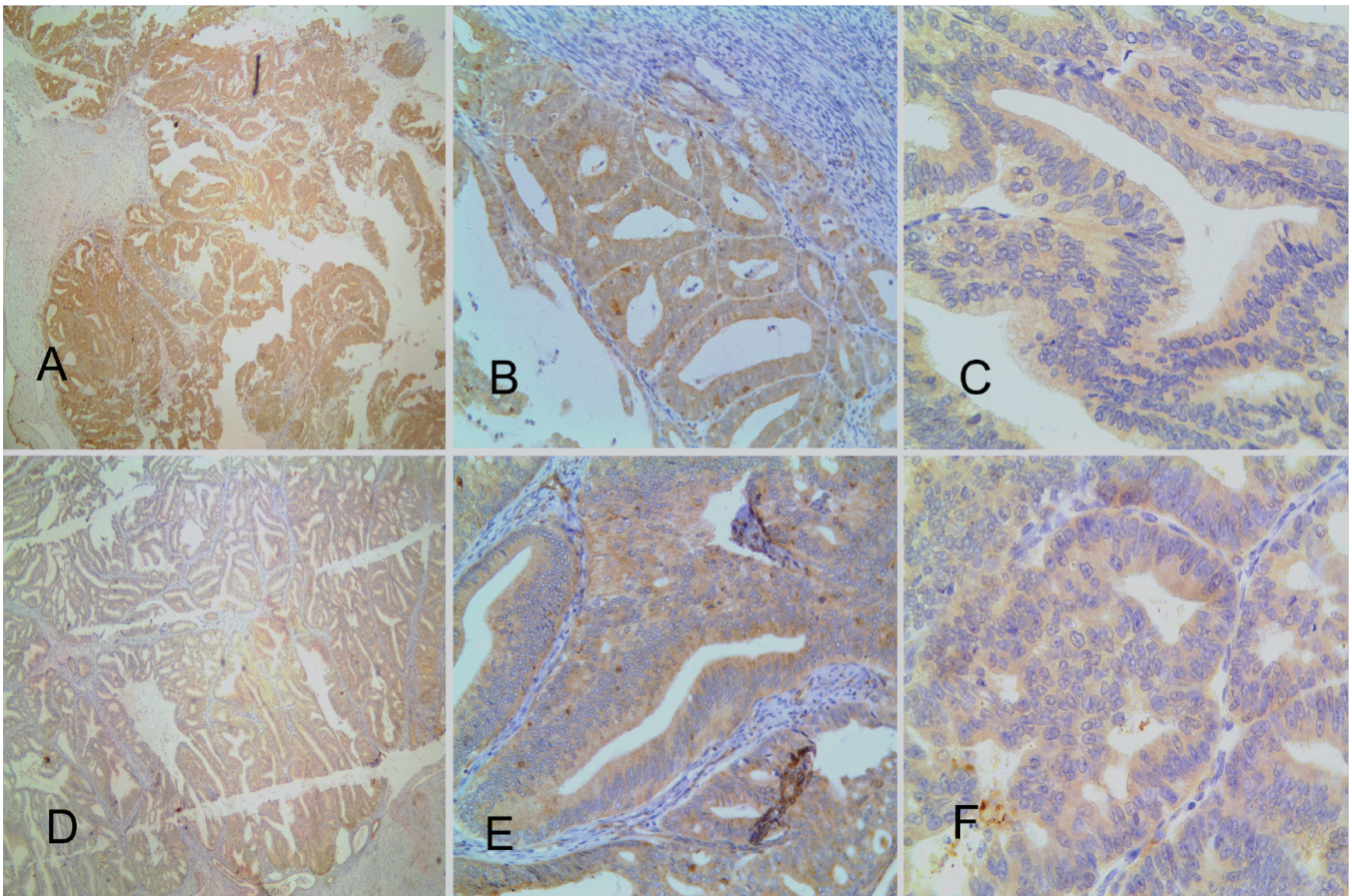


Fig. 1. Positive staining of SOX2 in EEC (A-C) and EOC (D-F) tissues. A, D, × 40; B, E, × 200; C, F, × 400.

Tumorigenesis in DPEEOC

were negatively expressed in NET and NOT tissues (Fig. 3), including one NOT tissue with faint yellow staining in less than 5% ovarian cells.

MiRNA-145, OCT4, and SOX2 expression in DPEEOC tissues

MiRNA-145 expression

The expression of miRNA-145 was significantly lower in EEC tissues from DPEEOC samples ($P < 0.001$, Fig. 4) compared to that in NET samples. However, the concentration of miRNA-145 was found to be higher in the EOC zones of DPEEOC samples compared to NOT samples ($P < 0.001$; Fig. 4). No significant differences in miRNA-145 expression were found between EEC and EOC in DPEEOC samples ($Z = -0.672$, $P = 0.502$; Fig. 4, Fig. 5). These results showed that the expression of miRNA-145 was the same between EEC and EOC tissues. Besides, we also discriminated that NET expressed higher levels of miRNA-145 than NOT ($Z = -3.780$, $P < 0.001$).

Expression of SOX2 and OCT4 in DPEEOC tissues

There were no significant differences in SOX2 expression between EEC and EOC tissues in DPEEOC samples ($t = -1.942$, $P = 0.052$; Fig. 6A). Additionally, no significant differences were observed in OCT4 expression between the two tissues ($t = -1.002$, $P = 0.329$; Fig. 6B). We also identified a correlation between SOX2 and OCT4 expression in EEC ($r = 0.449$, $P = 0.047$) and EOC ($r = 0.555$, $P = 0.011$) from DPEEOC samples. Although miRNA-145 can target both SOX2 and OCT4, our study did not find a strong correlation between miRNA-145 and SOX2 (EEC: $r = 0.192$, $P = 0.418$; EOC: $r = 0.146$, $P = 0.539$), or between miRNA-145 and OCT4 (EEC: $r = 0.123$, $P = 0.605$; EOC: $r = 0.261$, $P = 0.266$).

MiRNA-145, OCT4, and SOX2 expression in MDEEOC tissues

No significant differences in miRNA-145 ($Z = -0.447$, $P = 0.655$; Fig. 7), SOX2 ($t = -0.192$, $P = 0.852$; Fig. 8A), or OCT4 ($t = -0.893$, $P = 0.395$; Fig. 8B) expression were

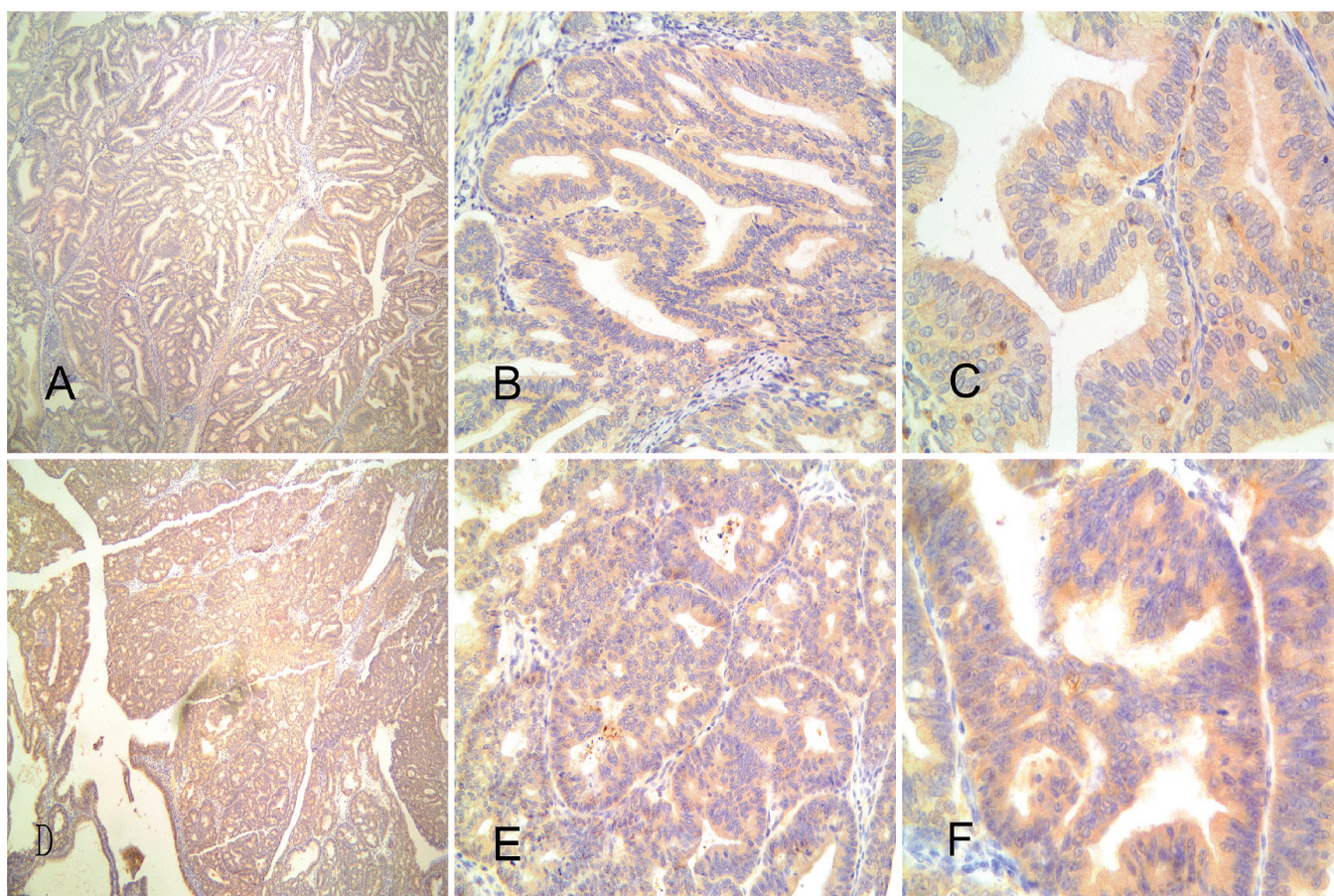


Fig. 2. Positive staining of OCT4 in EEC (A-C) and EOC (D-F) tissues. A, D, $\times 40$; B, E1, $\times 200$; C, F1, $\times 400$.

found between EEC and EOC tissues in MD_{EEOC} patients (EEC2 and EOC2, respectively). We also found a correlation between SOX2 and OCT4 expression in EEC ($r=0.770$, $P=0.009$) and EOC ($r=0.716$, $P=0.020$) from MD_{EEOC} samples. When compared to corresponding DP_{EEOC} tissues, MD_{EEOC} tissues showed higher expression levels of SOX2 and OCT4 (all

$P<0.001$). No difference of miRNA-145 expression was found between DP_{EEOC} and MD_{EEOC} (all $P>0.05$).

Discussion

Concurrent primary multiple gynecological cancers are not rare events in gynecological carcinomas. Of

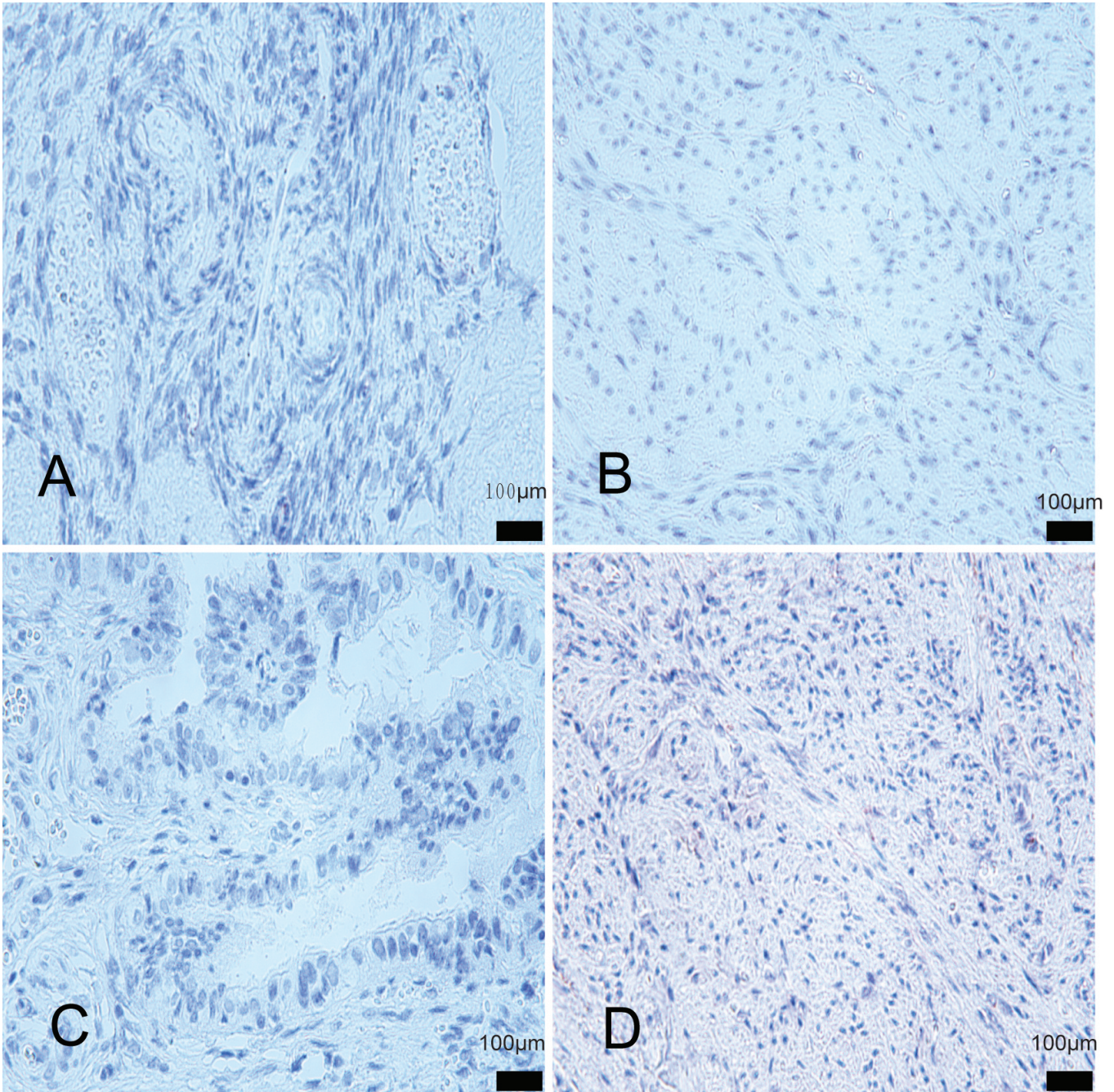


Fig. 3. SOX2 and OCT4 were negatively expressed in NET and NOT tissues. **A and C** showed SOX2 and OCT4 staining in NET tissues respectively. **B and D** showed SOX2 and OCT4 staining in NOT tissues, respectively.

Tumorigenesis in DPEEOC

these cancers, DPEEOC is the most common type. Most cases of DPEEOC can be diagnosed by investigating clinical and pathological characteristics. However, in some cases, it may be difficult to confirm whether the cancer is primary or metastatic. In the past decade, many studies have concentrated on the clonality of DEOC. Recently, several studies have provided robust evidence for the clonality of these tumors through gene sequencing. In a study by Chao et al. (2016), clonal relationship was demonstrated on double cancer sites of DEEOC. However, these clonal tumors were more often classified as metastatic. We do not completely agree with this conclusion, as we found that most of the patients in their study suffered from high-grade cancers. Anglesio et al. (2016) and Schultheis et al. (2016) each performed gene sequencing on pathologically diagnosed cases of DPEEOC and MDEEOC. Their results proved the model that both DPEEOC and MDEEOC were clonal in a given patient. Our study was performed in order to confirm clonality considering CSCs as a factor.

Cancer cells are generally poorly differentiated. They share many characteristics with embryonic cells, especially the capability for indefinite multiplication and differentiation. In DPEEOC lesions, endometrioid cancer cells were found at the sites of both tumor types. We hypothesized that these cells may have some connection with CSCs or CSC-like clusters. Currently, no studies have successfully identified the source of CSCs. However, studies have demonstrated that a terminally differentiated cell can restore its capacity for pluripotency (Byrne et al., 2007). Yamanaka and Blau. (2010) identified that artificially over-expressing key transcription factors can reprogram a somatic cell into a pluripotent one. CSCs are similar to ESCs in many ways. CSCs have the potential for self-renewal and differentiation. They play important roles in the

formation of many tumors, including endometrial adenocarcinomas and ovary carcinomas (Hubbard et al., 2009; McLean et al., 2011). Both SOX2 and OCT4 are crucial multipotent factors in ESCs and CSCs. Studies have reported that only SOX2 and OCT4 could induce fibroblasts to revert to pluripotent stem cells (Takahashi and Yamanaka, 2006). In cancers, they can control proliferation, apoptosis, invasion, and metastasis (Li et al., 2012; Santini et al., 2014; Ren et al., 2016a).

Furthermore, miRNAs are important for the functioning of stem cells. Genetic studies firstly identified the function of miRNAs in the maintenance of

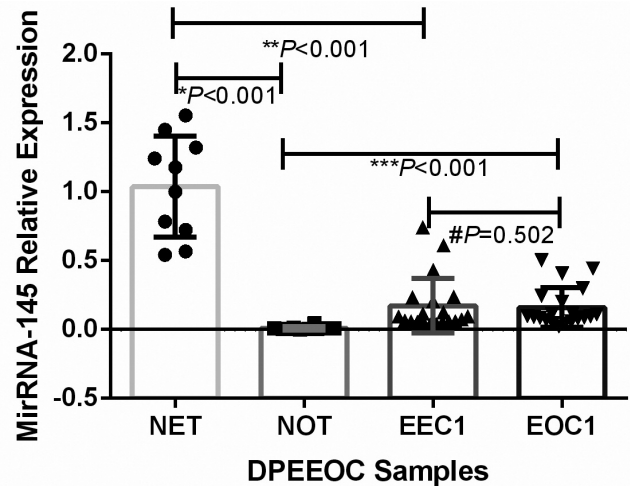


Fig. 4. The expression of miRNA-145 in DPEEOC tissues. The error bars indicate standard deviation. EEC1 referred to EEC in DPEEOC. EOC1 referred to EOC in DPEEOC.

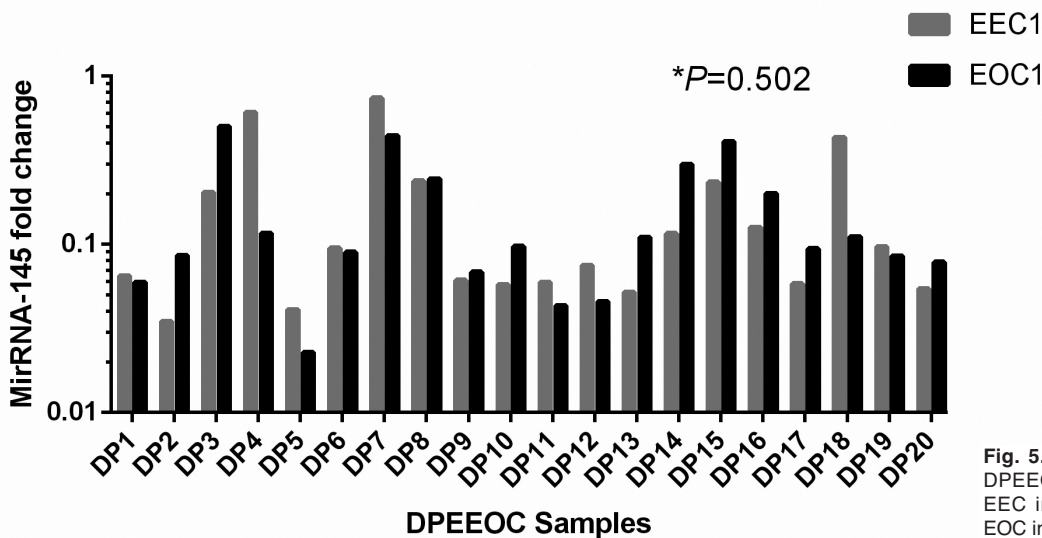


Fig. 5. Fold change of miRNA-145 in DPEEOC samples. EEC1 referred to EEC in DPEEOC. EOC1 referred to EOC in DPEEOC.

ESC populations and their pluripotency. MiRNAs are small non-coding RNAs that are 10-20 bp in length. Researchers have found that miRNAs have major roles in many cellular processes, including proliferation, differentiation, and apoptosis (Lin and Gregory, 2015). These miRNAs play potential roles in ESC activity, tumorigenesis, and stimulating inflammatory responses (Zeng et al., 2016). Recent studies have demonstrated that aberrant expression of miRNAs is related to the formation of tumors. This study focused on the expression of miRNA-145. Deregulation of miRNA-145 has been identified in ESCs and many cancers, including endometrial carcinomas and ovarian cancer (Wu et al., 2011; Chen et al., 2018). We searched the Targetscan

database and found a possible binding site for miRNA-145 on the 3-UTR of the mRNA sequences of both SOX2 and OCT4. SOX2 and OCT4 have very similar binding sites, which can both be targeted and mediated by miRNA-145. Xu et al. (2009) proved that miRNA-145 could suppress the pluripotency of ESCs by regulating OCT4 and SOX2 expression. They proved this by examining luciferase activity in HeLa cells. Wu et al. (2011) also showed the association between miRNA-145 and OCT4 by examining luciferase activity. Zou and his colleagues (Zou et al., 2016) demonstrated that miRNA-145 could bind to specific sites on SOX2 by using luciferase reporter assays.

To our knowledge, to date, no studies have been

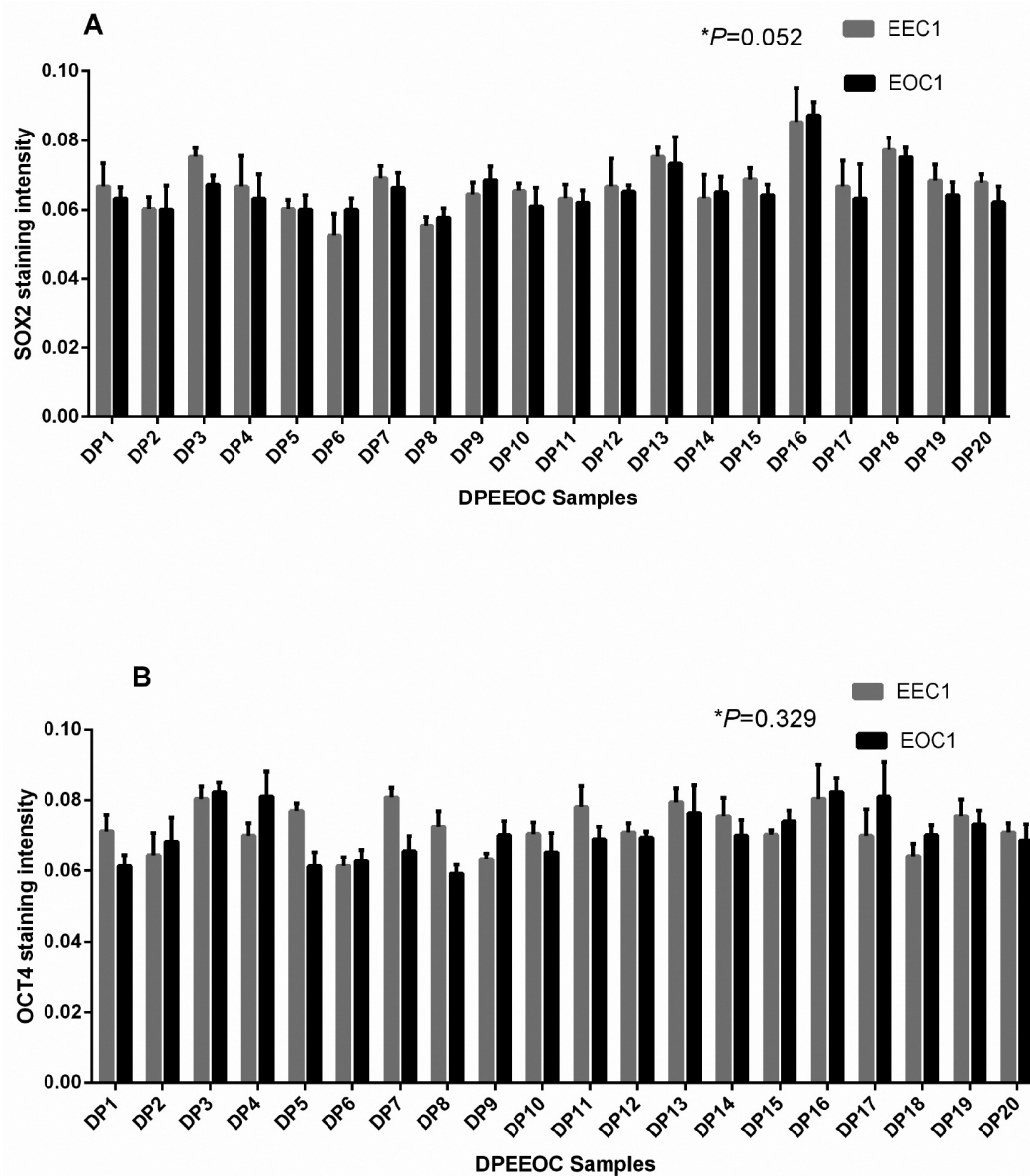


Fig. 6. Results of quantitative analysis for IHC staining of SOX2 (A) and OCT4 (B) in all DPEEOC tissues. The error bars reveal standard deviation. EEC1 referred to EEC in DPEEOC. EOC1 referred to EOC in DPEEOC.

Tumorigenesis in DPEEOC

performed on the expression of SOX2 and OCT4 in DPEEOC. Our study showed SOX2 and OCT4 were positively expressed in all cancerous FFPE tissues, but not in NET or most NOT. Only one NOT was slightly stained by OCT4. Pitynski et al. (2015) performed IHC on single EEC lesions and observed that SOX2 and OCT4 were expressed in most lesions. In addition, they found that SOX2, but not OCT4, showed a correlation with tumor grading. However, we did not find a similar grading correlation because most of the cancer tissues in our study were mainly low-grade. In serous ovarian carcinomas, studies have shown that both SOX2 and OCT4 are over-expressed (Zhang et al., 2010; Bareiss et al., 2013). To our knowledge, no similar research has been performed on endometrioid ovarian carcinomas. Some studies have investigated the function and mechanism of SOX2 and OCT4 on endometrial and ovarian carcinomas. These studies demonstrated that SOX2 can influence the prognosis of endometrial cancer by blocking the P21 expression pathway (Yamawaki et

al., 2017). Wu et al. (2011) reported that miRNA-145 could repress OCT4 and promote the differentiation of endometrial cancer cells. Wang et al. (2014) reported that SOX2 can promote the invasion and migration of ovarian cancer cells by targeting Src kinase. Lin and his colleagues demonstrated that MiR-26b/KPNA2 can inhibit ovarian tumor proliferation and metastasis by decreasing OCT4 expression (Lin and Gregory, 2015). However, many mechanisms remain unclarified in this regard. In the current study, IHC revealed that SOX2 and OCT4 might play a role in DPEEOC tumorigenesis.

The expression of miRNA-145 was also measured in all samples. Our report is the first to compare the expression between double cancer sites in DPEEOC patients. Deregulation of miRNA-145 was found in all cancer tissues investigated in this study. We found that miRNA-145 was expressed at lower levels in EEC tissues of DPEEOC compared to NET. However, its expression was increased in EOC tissues of DPEEOC when compared to NOT. Similar results were also found

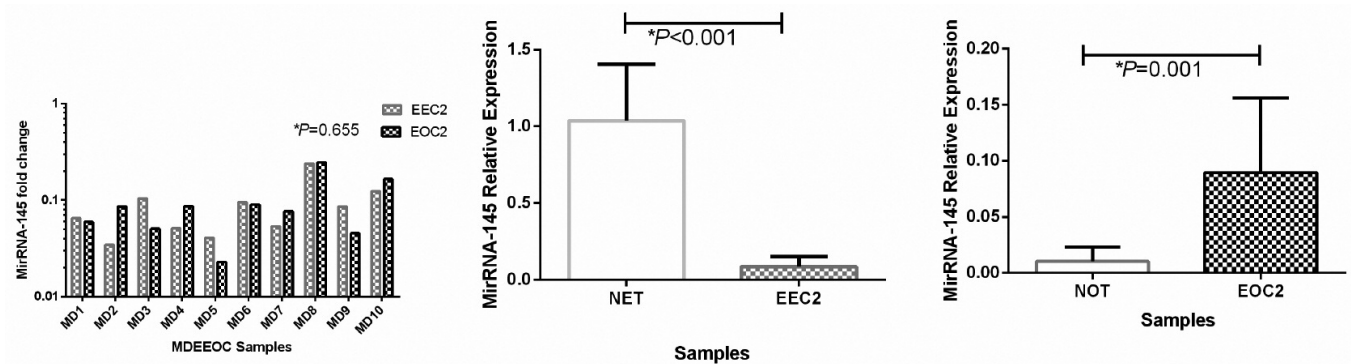


Fig. 7. Fold change of miRNA-145 in MDEEOC samples. EEC2 referred to EEC in MDEEOC. EOC2 referred to EOC in MDEEOC.

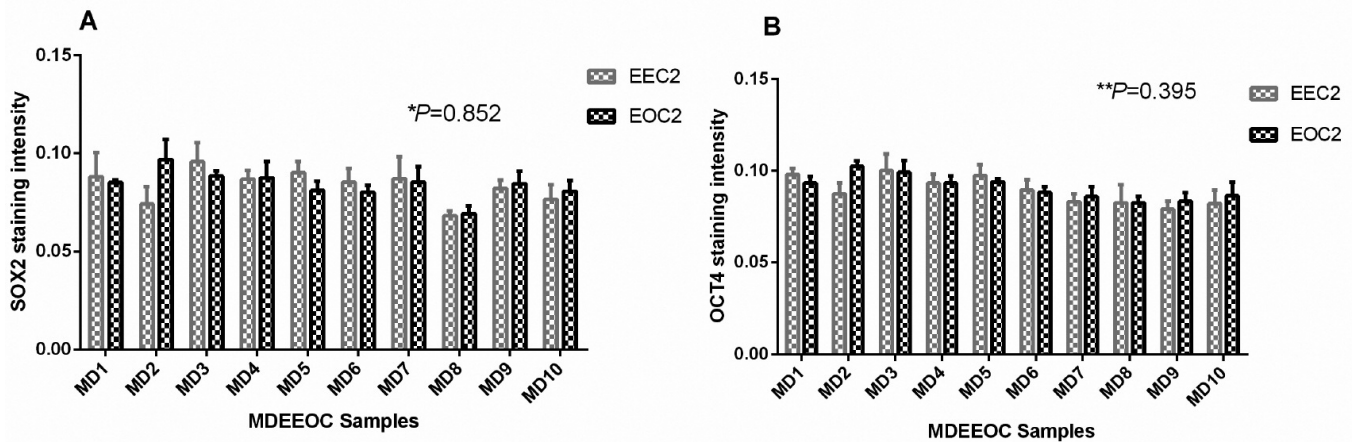


Fig. 8. Results of quantitative analysis for immunohistochemistry staining of SOX2 and OCT4 in all MDEEOC tissues. A showed the expression of SOX2. B showed the expression of OCT4. The error bars reveal standard deviation. EEC2 referred to EEC in MDEEOC. EOC2 referred to EOC in MDEEOC.

in MDEEOC tissues. These results seemed to contradict several previous studies. MiRNA-145 expression was found to be decreased in many cancers, including endometrial and non-endometrioid ovarian carcinomas. Wu et al. demonstrated that miRNA-145 expression was reduced in serous or clear cell ovarian carcinomas. However, no increase in the expression of miRNA-145 was found in endometrioid ovarian lesions (Wu et al., 2013). The reasons for this are unclear; other diseases or mechanisms may have contributed to the contradictory results in previous studies. For example, miRNA-145 was reported to have a higher expression in endometriosis lesions (Zheng et al., 2014). However, EOC was not always comorbid with endometriosis. Further studies are required to determine the reasons for this effect. However, the hypothesis that miRNA-145 functions as a tumor suppressor may be not accurate. Additionally, we discovered that miRNA-145 expression was higher in NET samples than in NOT samples. This may be because miRNA-145 is an organ-related miRNA (Landgraf et al., 2007).

To demonstrate the effects of miRNA-145 on SOX2 and OCT4 in DPEEOC, we performed statistical comparisons. However, no correlations were found in either of the two cancer tissues. Interestingly, we discovered a correlation between the expression of SOX2 and OCT4 in DPEEOC tissues. No significant differences in SOX2, OCT4, and miRNA-145 levels were found in the EOC and EEC tissues in a given DPEEOC patient. Previous studies have shown that the differential expression of all three molecules can have remarkable effects. OCT4 and SOX2 are critical to establishing the ESC state. In ESCs, different doses of OCT4 may govern the fate of different cells and mediate the direction of differentiation (Niwa et al., 2000). Masui et al. (2007) demonstrated that SOX2 was a vital factor in modulating OCT4 expression and maintaining the features of ESCs. Changes of SOX2 and OCT4 expression can regulate the differentiation and dedifferentiation of different kinds of embryonic cells. A fine balance of OCT4 or SOX2 expression is also critical for maintaining the function of CSCs. These two factors could take effect by formatting OCT4 and SOX2 enhancers, or by being regulated by the same molecules. The similar expression levels of SOX2 and OCT4 indicated that similar cancer subpopulations (such as CSCs) may be found, leading to the same tumor types (Ibrahim-Hashim et al., 2017). This expression relation might explain the embryonic origin of cancer development (Ratajczak et al., 2010). Iskender and his colleagues (Iskender et al., 2016) demonstrated that different combinations of four transcription factors: SOX2, OCT4, KLF4, and c-myc could generate six sub-clones in bladder T24 cancer cells. In each clone, similar morphological characteristics and cell shapes existed. Cell types co-expressing SOX2 and OCT4 were found to be related (Whyte et al., 2013). Xu et al. (2009) demonstrated that undifferentiated human ESCs expressed relatively low levels of miRNA-145. With the

ESCs differentiating into embryonic organs, its expression was significantly increased. Quantitative expression of miRNA-145 is also related with the stem cell state. Ectopic deregulation of miRNA-145 could induce morphological changes of cells by targeting many genes (Yamada et al., 2013). Our study demonstrated similar expression of SOX2, OCT4 and miRNA-145 in the two cancer tissues of DPEEOC. Besides, co-expressed and positively correlated SOX2 and OCT4 were also discovered. By IHC staining, we demonstrated that SOX2 and OCT4 were mainly expressed in the cytoplasm of endometrioid endometrial and ovarian cancer cells in DPEEOC. Together, these results support that there exist similar undifferentiated subpopulations. In DMEEOC, one cancer was transferred from another cancer. Since its discovery, clonality has been accepted as a contributing factor. In MDEEOC, similar expression levels of miRNA-145, OCT4 and SOX2 were found (Figs. 7, 8). Co-expressed and positively related SOX2 and OCT4 were also demonstrated. Although our results could not fully demonstrate the clonality of DPEEOC, the results were in accordance with the conclusions proved by Anglesio et al. (2016), Schultheis et al. (2016) and Chao et al. (2016) regarding the clonality of cancer cells.

When comparing between DPEEOC and MDEEOC, we discovered that DPEEOC tissues expressed lower levels of SOX2 and OCT4 compared to the corresponding MDEEOC sections. This may have some implication in distinguishing DPEEOC and MDEEOC. Besides, many studies have demonstrated that the two factors are correlated with prognosis in many types of cancers. Further studies are needed to validate the relationship between SOX2 and OCT4 expression and the prognosis of DEEOC patients.

Even in consideration of the limited samples in our study and restricted molecules, we can say that our results support the the clonality of DPEEOC. Furthermore, SOX2 and OCT4 expression analysis may have some implications in DPEEOC and MDEEOC diagnosis, especially for those undetermined ones. That will be helpful in improving treatment strategies for those patients. More studies with more patients and more in-depth experiments are required to further demonstrate our results.

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Tumorigenesis in DPEEOC

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