Exploration on correlation of high DLX2 expression with poor prognosis and cellular proliferation in epithelial ovarian cancers

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Objective: It has been found that overexpression of distal-less homeobox 2 (DLX2) is closely correlated with multiple cancers. However, the role of DLX2 in the pathogenesis of ovarian cancers is not known. This study was designed to explore the mechanism of action of DLX2 on cellular proliferation and apoptosis in epithelial ovarian cancers (EOCs).

Methods: A total of 119 EOC tissue specimens were analyzed immunohistochemically, and the correlation between DLX2 expression level and clinicopathological characteristics was determined. Moreover, western blot method was used to measure DLX2 protein in EOC specimen of different grades and EOC cell lines and explore its molecular mechanism of action. Kaplan-Meier survival analysis revealed that overexpression of DLX2 was obviously correlated with an adverse clinical outcome in EOCs (P < 0.01). Results: DLX2 expression level had an obvious association with histopathological grade, FIGO stage, ascites and ki-67 expressional level in EOC patients and DLX2 exerted a crucial effect in regulating cellular proliferation in EOCs. Knocking down DLX2 using shRNA-DLX2 reduced the proliferation and enhanced the apoptosis in EOC cells. Conclusion: DLX2 might act as a new prognostic indicator and have an important value for molecular targeted therapeutic drugs in EOCs.

Keywords
Epithelial ovarian cancer (EOC), DLX2, Proliferation

1. Background

Epithelial ovarian cancers (EOCs) are most commonly seen in females, which exhibit the highest mortality among all gynecology malignant tumors around the world [1]. Although surgical treatment, chemotherapy or radiotherapy has obviously promoted qualities of life in EOC patients, the early diagnostic and curative ratios remain low [2]. Therefore this is still a big challenge in the treatment of EOC patients. It is important to establish a new strategy for the diagnosis and treatment of EOCs to identify novel molecular mechanisms during the disease development.

Distal-less homebox (DLX) genes, a homolog of Drosophila distal-less, compose a family of homebox transcription factors participating in regulation of cellular differentiation and morphogenesis. Some evidences have showed that the DLX gene family plays a vital role in regulation of embryogenesis, tissue homeostasis, cellular cycle and apoptosis [3]. Members of DLX gene family exert critical effects on cancer progression. For example, the inhibition of DLX4 enhances the apoptosis of choriocarcinoma cells [4]. Besides, researchers also found that up-regulated expression of DLX4 can promote migration, invasion and metastasis of tumor cells [5]. DLX5 can enhance cellular proliferation in tumors by transcriptionally regulating MYC [6]. Moreover, it has been found that DLX5 is closely correlated with cellular proliferation in ovarian cancers [7]. Those findings indicated that DLX gene family might participate in cancer development. It has been reported that one of DLX gene family members-DLX2 can activate mitogenic epidermal growth factor receptor (EGFR) signaling through direct induction of EGFR ligand betacellulin expression [8]. In addition, it has also been reported that DLX2 is abnormally detected in many human carcinomas such as, prostate carcinoma, hepatocellular carcinoma and breast carcinoma [9–11]. However, the effect of DLX2 on the progression of EOCs remain unexplored In our study, IHC was firstly applied to explore the correlation of DLX2 with clinicopathologic indicators in EOC patients. The results revealed that a high-level expression of DLX2 could predict adverse clinical outcome in EOC patients. Thereafter, short hairpin RNA (shRNA) was used to knockdown the expression of DLX2 to decrease the specific effect of DLX2 on EOC cells. CCK-8 and apoptosis assay were used for further exploration. Short hairpin RNA (shRNA) was used to knock down the expression of DLX2 to decrease the specific effect of DLX2 on EOC cells. It was found that low DLX2 expression level could decrease proliferation and promote apoptosis in EOC cells. The above results indicated that DLX2 participated in the pathogenic process of EOCs and might predict a adverse clinical outcome in EOC patients.
Table 1. DLX2 and Ki-67 Expression and Clinicopathological Parameters in 119 OC Specimens.

| Parameters                      | DLX2        | P-value | χ²    | Ki-67       | P-value | χ²    |
|--------------------------------|-------------|---------|-------|-------------|---------|-------|
|                                | Low | High |       | Low | High |       |
| Age (years)                    |     |      | 0.354 | 0.860 | 0.659 | 0.195 |
| < 50                           | 11  | 24   |       | 14  | 21   |       |
| > 50                           | 34  | 50   |       | 30  | 54   |       |
| Histological grade             |     |      | 0.038* | 6.561 | < 0.001* | 30.622 |
| Well                           | 5   | 2    |       | 7   | 0    |       |
| Moderate                       | 17  | 19   |       | 24  | 14   |       |
| Poor                           | 23  | 53   |       | 15  | 61   |       |
| FIGO stage                     |     |      | 0.046* | 8.004 | 0.265 | 3.969 |
| I                              | 27  | 25   |       | 24  | 28   |       |
| II                             | 4   | 9    |       | 3   | 10   |       |
| III                            | 13  | 36   |       | 16  | 33   |       |
| IV                             | 1   | 4    |       | 1   | 4    |       |
| Menopause                      |     |      | 0.457 | 0.554 | 0.168 | 1.902 |
| Absence                        | 14  | 28   |       | 29  | 23   |       |
| Presence                       | 31  | 46   |       | 25  | 52   |       |
| Ascites                        |     |      | 0.009* | 6.860 | 0.037* | 4.365 |
| Absence                        | 34  | 38   |       | 32  | 40   |       |
| Presence                       | 11  | 36   |       | 12  | 35   |       |
| Metastasis to lymph node       |     |      | 0.077 | 3.133 | 0.228 | 1.450 |
| Absence                        | 40  | 56   |       | 38  | 58   |       |
| Presence                       | 5   | 18   |       | 6   | 17   |       |
| Metastasis to other organ      |     |      | 0.076 | 3.143 | 0.006* | 7.447 |
| Absence                        | 27  | 32   |       | 29  | 30   |       |
| Presence                       | 18  | 42   |       | 15  | 45   |       |
| Cancer cells in ascites        |     |      | 0.110 | 2.557 | 0.017* | 5.743 |
| Absence                        | 38  | 53   |       | 39  | 52   |       |
| Presence                       | 7   | 21   |       | 5   | 23   |       |
| Ki-67                          |     |      | 0.004* | 8.310 |       |       |
| Low                            | 24  | 20   |       |       |       |       |
| High                           | 21  | 54   |       |       |       |       |

Statistical analyses were performed by Pearson χ² test. * P < 0.05 was considered significant.

2. Materials and methodology

2.1 Subject and specimen selection

A total of 119 EOC tissue specimens were acquired from the patients treated in the Obstetrics and Gynecology Department of the Second Affiliated Hospital of Nantong University. This study achieved an approval from the medical ethics committee of the above hospital, and all subjects had signed a written informed consent form. Major clinicopathological characteristics of patients such as age, cancer classification, FIGO stage, menstrual status, lymph node metastasis and organic metastases, ascitic fluid, cancer invasiveness and overall survival (OS) were presented in Table 1.

All specimens were quickly frozen in liquid nitrogen and stored at -80 °C until being used for Western blotting detection.

2.2 Immunohistochemistry staining

All slices with 5 MM thickness were prepared and then placed on 10% polycline-coated glass slides. The slices were rinsed twice with xylene (15 min each time) and then rehydrated in graded ethanol. Thereafter the slice were added with antibodies such as DLX2 (diluting ratio 1 : 200, Invitrogen, Carlsbad, CA, USA), Ki-67 (diluting ratio, 1 : 500, Invitrogen, Carlsbad, CA, USA) and cultivated for 2 h at indoor temperature; subsequently the slices were rinsed thrice with PBS; the signal development was performed using diaminobenzidine; all slices were dyed with 20% hematoxylin-eosin; finally all slices were dried, cleaned and assessed.

At least 500 cells or more per visual field were observed to calculate the label index, which was the ratio of positively dyed cells to total cells. The numbers of Dlx2 and Ki67 positive cells were calculated, and the proportion of positive cells was calculated. The dying status was evaluated according to the proportion of positive cancer cells: 0 point indicated negative result and positively dyed cells < 5%; 1 point indicated 5–25% positively dyed cells; 2 points indicated 25–50% positively dyed cells; 3 points indicated 50–75% positively dyed cells and 4 points indicated > 75% positively dyed cells. The immunoreactivity intensity was evaluated for each
cancer slice: 0 point indicated negative staining; 1 point indicated weak intensity; 2 points indicated moderate intensity; 3 points indicated strong intensity. The scores for percentage and intensity were multiplied to obtain the final scores 0, 1, 2, 3, 4, 6, 9, or 12. Statistical analysis showed that a score ≤ 4 points indicated low expression, and a score > 4 points indicated a strong overexpression [12].

2.3 Western blotting assay

EOC tissues and cells were analyzed with Western blotting assay. The tissues were lysed in lysis buffer comprising protease inhibitor mixture solution. The lysates were centrifuged at 12,000 rpm for 20 min at 4 °C. Thirty mcg total proteins were analyzed using SDS-PAGE and then transferred onto a PVDF membrane (Immobilon, Millipore, Biirica, MA, USA), which was immersed in blocking buffer containing skimmed milk powder and cultivated with appropriate primary and secondary antibodies. Enhanced chemiluminescence (ECL; Pierce Company, Rockford, IL, USA) was used to measure protein signals. The antibodies included anti-rabbit (Santa Cruz, Delaware Ave, CA, USA, diluted at 1 : 500), caspase-3 (Cell Signaling Technology, Beverly, MA, USA), which was immersed in blocking buffer containing 100 µL medium and cultivated overnight. CCK-8 reagents (Dojindo, Kumamoto, Japan) were added into a subset of wells under different treatments and the cells were cultivated for 1 h at 37 °C. Finally, an appropriate amount of solution reaction was taken to detect the absorbance at 490 nm.

2.7 Transfection of short hairpin RNA

Short hairpin RNAs (shRNAs) were provided by Shanghai Genechem Co. Ltd (Shanghai, China). DLX2 gene expression was decreased by shRNA d ext targetting the sequence, designated shRNA-1: 5'-GAACGGGAAGCCAAAGAAA-3', designated shRNA-2: 5'-CTGAAAATTCGGAATAGTGA-3' and, designated shRNA-3: 5'-ATATGCACTCGACGACAGAT-3'. The shRNA 5'-UUCUCGGAACGUGUCAGCU-3' was used as a negative control (Neg) of mRNAs. DLX2 shRNAs were transfected into the cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) referring to the procedure described in the manual.

2.8 Apoptosis analysis

The cultured cells were co-transfected with control-shRNA and DLX2-shRNA, respectively. Thereafter, the cells were dissociated into single cell suspension with EDTA-free trypsin and rinsed twice using PBS. EOC cells were dyed using Annexin-V-FITC/PI, and then the cellular apoptosis was quantified by a flow cytometer.

2.9 Statistical treatment

The data were statistically analyzed by SPSS 10.0 (SPSS Inc, Chicago, IL, USA). The correlations of DLX2 and Ki-67 with clinicopathological characteristics were determined by chi-square test. The survival was analyzed using Kaplan-Meier survival curve and the log-rank test. Cox model was used to conduct multivariate analysis, and P < 0.05 indicated an obvious difference.

3. Results

3.1 Association between DLX2 expression level and survival of EOC tissues and cells

To investigate the clinicopathological effect of DLX2 on EOCs, immunohistochemistry analysis was firstly conducted to examine DLX2 protein expression level in 119 EOC specimens. DLX2 and Ki-67 expression level in EOCs of various histopathological grades were presented in Fig. 1. DLX2 and Ki-67 were not or lowly expressed in low grade EOC specimens (Fig. 1A,D), meanwhile it was confirmed that DLX2 and ki-67 were strongly expressed in poor grade EOC specimens (Fig. 1C,F). The results indicated that DLX2 is closely correlated with prognosis in EOC patients.

To confirm this viewpoint, the expressions of DLX2 in additional specimens were detected using western blotting (three normal ovarian tissue specimens and three different specimens for each of three grades of EOC tissue specimens). The findings of this study revealed that the expression level of...
Fig. 1. Immunohistochemical detection of DLX2 and Ki-67 expressions in EOCs with various histopathological grades. A and D indicated DLX2 and Ki-67 expressions in stage I EOCs, respectively. B and E indicated DLX2 and Ki-67 expressions in stage II EOCs, respectively. C and F indicated DLX2 and Ki-67 expressions in stage III EOCs, respectively.

Fig. 2. Expressional statuses of DLX2 in EOCs. (A) Western blotting detection of DLX2 expressions in EOCs with different histopathological grades. (B) Western blotting indicated DLX2 protein expressions in 4 kinds of EOC cells. (C) The bar chart indicated a ratio of DLX2 to GAPDH measured by a densitometer. The results were presented as mean ± SEM. GAPDH was applied as a loading control. Each experiment was performed thrice.

DLX2 was significantly higher in tumors in comparison with normal ovarian tissues (Fig. 2A). Thereafter, a difference in DLX2 expression level was detected among 4 EOC cell lines, indicating that DLX2 was highly expressed in SKOV3 cells (Fig. 2B). Therefore, SKOV3 cell line was used for subsequent studies.

3.2 Association between DLX2 expression and clinicopathological characteristics of EOCs

In order to explore the pathogenic effect of DLX2 in EOCs, clinicopathological indicators were detected to assess the relationship between DLX2 and Ki-67 expressions. The histopathological characteristics of patients were showed in...
The study results revealed that DLX2 expression level had an obvious association with histopathological grade, FIGO stage, ascites and Ki-67 expression level in EOC patients, but had no association with other prognosis indicators. In addition, Ki-67 expression had a close association with histopathological grade, ascites, lymph-node metastases and ascetic cancer cells. Thereafter, scatter plot further indicated an association of DLX2 with Ki-67. As shown in Fig. 3, DLX2 positive cancer cells accounted for 8-93%, and Ki-67 positive cancer cells accounted for 9-98%. Therefore, it could be concluded that the expression level of DLX2 was close to that of Ki-67 in EOCs, which indicated that EOC tissues with a poorer histopathological grade had higher expression levels of DLX2 and Ki-67 compared with those with better and moderate histopathological grades. The above results revealed a close association of DLX2 expression level with Ki-67-defined proliferative activity ($P < 0.01$; Fig. 3).

3.3 Prognosis effect of DLX2 expression

The OS of 119 EOC patients was analyzed using immunohistochemistry, the results were presented in Table 2. Only 19 of 74 (25.7%) EOC patients with a high DLX2 expression still survived, while 31 of 45 (68.9%) EOC patients with a low DLX2 expression still survived at 5 years. All influencing factors of survival of EOC patient were analyzed separately, and the results reveal that the histopathological grade, FIGO stage, organic metastases, DLX2 and Ki-67 expression levels obviously affected the OS (Table 2). Furthermore, Kaplan-Meier analysis was conducted to determine the association of DLX2 expression with OS in EOC patients, the results indicated that a higher DLX2 expression level had a close association with a poorer OS ($P < 0.01$; Fig. 4). Therefore, a multivariate analysis was conducted by Cox model, the results revealed that DLX2 was an independent prognosis index for OS in EOC patients (Table 3).

3.4 Expression of DLX2 in proliferating EOC cells

This study revealed that a higher DLX2 expression level might be correlated with oncogenesis in EOCs. To further confirm the role of DLX2 in cell-cycle progression, the cell cycle was analyzed after serum deprivation and re-feeding with serum, respectively. Fig. 5F indicated G1 phase cell cycle arrest in EOCs after 48 hours of serum deprivation, and cell cycle progression from G1 to S phase in EOCs after re-feeding with serum, which indicated DLX2 expression level was increased after re-feeding with serum. In addition, CDK2 and Cyclin A were lowly expressed at G0/G1 phase and highly expressed at S phase after serum stimulation (Fig. 5G). These studies suggested that DLX2 exerted a crucial effect in regulating cellular proliferation in EOCs.

3.5 Effect of DLX2 on EOC cells

The effect of DLX2 on the progression of EOC cells was investigated through shRNA knockdown of DLX2 in SKOV3 cells, which were transiently transfected with DLX2-shRNA and control-shRNA, respectively. Fig. 6A showed that DLX2 expression level in the DLX2 shRNA-transfected cells was obviously decreased in comparison with that in the control-shRNA-transfected cells It was also confirmed that DLX2-shRNA-3 showed the greatest knockdown efficiency among three shRNAs (Fig. 6B). Therefore, DLX2-shRNA-3 was applied in the following experiments.

In this study, CCK-8 assay was conducted to assess the effect of DLX2 on cellular proliferation of EOCs, and the results indicated that the cellular proliferation in DLX2-shRNA-transfected EOC cells was obviously decreased in comparison with that in control-shRNA transfected EOC cells (Fig. 6C). Therefore, the invasive ability of DLX2-shRNA-transfected EOC cells were obviously decreased, while the proliferat-
Fig. 5. Expressions of SGTA and cell cycle-related indicators in EOC cells. (A, B, C, D, E) The cell cycle in SKOV3 cells was analyzed by Flow cytometry, the results indicated that the cells were staying at G1 phase after 48 hours of serum starvation, and then passed from G1 phase to S phase after being cultured in MEM supplemented with 10% FBS. (F) The bar chart revealed a ratio of S to G0/G1 phase cells. (G) SKOV3 cells were synchronized after 48 hours of serum starvation or after serum stimulation, the cellular lysates were evaluated by Western blotting using antibodies against CDK2 and Cyclin A. GAPDH served as a loading control. The results were presented as means ± SEM (n = 3, *, #: P < 0.05, in comparison with control group after 48 hours of serum starvation). S: serum starvation; R: serum release.

3.6 Knockdown of DLX2 induces apoptosis in EOC cells

Because some evidences showed that DLX2 can regulate cellular apoptosis [13], whether DLX2 could affect the EOC cellular apoptosis was explored in this study. At first, the effect of DLX2 on the apoptosis of EOC cells was determined by a flow cytometer. The results indicated that the apoptosis was greater in DLX2-shRNA-transfected EOC cells in comparison with the control group (Fig. 6G). Furthermore, the expression of apoptosis index caspase 3 in EOC cells transfected with DLX2-shRNA or control shRNA were detected. The results suggested that caspase 3 was highly expressed in DLX2-shRNA-transfected EOC cells (Fig. 6H). All of these studies revealed that DLX2 might be an essential factor in regulating the apoptosis of EOC cells.

4. Discussion

EOCs are caused by various molecular factors, and the death rate of EOC patients is still very high. Hence, it is crucial to investigate the molecular pathogenesis of EOCs, and this study aimed to explore the effect of DLX2 in the progression of EOCs.

A previous study has indicated that DLX2 protects cells from transforming growth factor β (TGF β) induced cell-cycle arrest and apoptosis [13]. Furthermore, previous studies have confirmed the effect of DLX2 in multiple human carcinomas such as breast carcinoma, prostate carcinoma and hepatocellular carcinoma [9–11]. It was found that DLX2 has a close association with clinicopathological characteristics of cancers. In this study, the correlation of DLX2 with the clinicopathological characteristics of EOCs was investigated, the results indicated that a high expresional level of DLX2 had a obvious correlation with histopathological grade, FIGO stage, ascites and Ki-67 in EOCs. In addition, DLX2 expresional level was positively associated with Ki-67 expresional level it was found that histopathological grade was closely
Fig. 6. Knocking down DLX2 suppressed he cellular proliferation and induced cell cycle arrest at G2/M phase and enhanced cellular apoptosis in EOCs. (A) CDK1 expression was measured by Western blotting at 48 h after shRNA transfection of SKOV3 cells, and the results indicated that CDK1-shRNA-3 showed the greatest knockdown efficiency among three shRNAs. (B) The bar chart indicated a ratio of DLX2 to GAPDH measured by a densitometer. The results were presented as mean ± SEM (P < 0.01, in comparison with the control group). (C) Cell growth rate of SKOV3 cells was measured. The results were presented as mean ± SEM; each experiment was repeated thrice (**P < 0.01, in comparison with the control group). (D) PCNA and CDK2 expressions in SKOV3 cells wherein DLX2 was knocked down were analyzed using Western blotting. (E) At 48 h after transfection, the cells were dyed by PI, and DNA content was analyzed by a flow cytometer. (F) The bar chart indicated a ratio of S to G0/G1 phase cells. *, #: P < 0.05 indicated a obvious difference. (G) apoptosis status in SKOV3 cells transfected with or without DLX2-shRNA was detected by a flow cytometer. (H) Caspase 3 expressions in control-transfected cells, and DLX2-shRNA were detected by a flow cytometer.
Table 2. Survival Status and Clinicopathological Parameters in 119 EOC Specimens.

| Parameters          | Total | Survival status | P-value | χ²  |
|---------------------|-------|-----------------|---------|-----|
|                     |       | Dead, n (%)     | Alive, n (%) |     |     |
| Age (years)         | 0.080 | 3.064           |          |     |     |
| < 50                | 35    | 16              | 19       |     |     |
| > 50                | 84    | 53              | 31       |     |     |
| Histological grade  | 0.007*| 9.793           |          |     |     |
| Well                | 7     | 1               | 6        |     |     |
| Moderate            | 36    | 17              | 19       |     |     |
| Poor                | 76    | 51              | 25       |     |     |
| FIGO stage          | < 0.001*| 21.258         |          |     |     |
| I                   | 52    | 18              | 34       |     |     |
| II                  | 13    | 11              | 2        |     |     |
| III                 | 49    | 36              | 13       |     |     |
| IV                  | 5     | 4               | 1        |     |     |
| Menopause           | 0.091 | 2.862           |          |     |     |
| Absence             | 43    | 20              | 23       |     |     |
| Presence            | 77    | 49              | 28       |     |     |
| Ascites             | 0.296 | 1.090           |          |     |     |
| Absence             | 72    | 39              | 33       |     |     |
| Presence            | 47    | 30              | 17       |     |     |
| Metastasis          | 0.085 | 2.970           |          |     |     |
| to lymph node       |       |                 |          |     |     |
| Absence             | 96    | 52              | 44       |     |     |
| Presence            | 23    | 17              | 6        |     |     |
| Metastasis          | < 0.001*| 14.384         |          |     |     |
| to other organ      |       |                 |          |     |     |
| Absence             | 59    | 24              | 35       |     |     |
| Presence            | 60    | 45              | 15       |     |     |
| Malignant tumor     | 0.099 | 2.717           |          |     |     |
| Cells in ascites    |       |                 |          |     |     |
| Absence             | 91    | 49              | 42       |     |     |
| Presence            | 28    | 20              | 8        |     |     |
| DLX2                | < 0.001*| 21.449         |          |     |     |
| Low                 | 45    | 14              | 31       |     |     |
| High                | 74    | 55              | 19       |     |     |
| Ki-67               | 0.004*| 8.354           |          |     |     |
| Low                 | 43    | 18              | 26       |     |     |
| High                | 76    | 51              | 24       |     |     |

Statistical analyses were performed by Pearson χ² test.

Table 3. Contribution of Various Potential Prognostic Factors to Survival by Cox Regression Analysis in 119 Specimens.

| Parameters          | Relative ratio | 95% Confidence interval | P-value |
|---------------------|----------------|-------------------------|---------|
| Histological grade  | 1.635          | 0.968–2.762             | 0.066   |
| FIGO stage          | 1.315          | 0.923–1.875             | 0.130   |
| Metastasis to other organ | 1.501  | 0.740–3.042             | 0.260   |
| Ki-67               | 0.911          | 0.479–1.736             | 0.778   |
| DLX2                | 0.325          | 0.173–0.611             | < 0.001*|

Statistical analyses were performed by Cox test. *P < 0.05 was considered significant.

Correlated with OS of EOC patients. Kaplan-Meier survival analysis indicated that a higher DLX2 expression level predicted a poorer OS in EOC patients. Furthermore, multivariate analysis indicated that DLX2 was associated with high-risk clinical parameter and served as an independent prognosis index for OS in EOC patients. This study showed that DLX2 was a significant gene affecting the progression of EOCs.

Furthermore, it was determined in this study that DLX2 exerted an effect on cellular proliferation in EOCs. Cell-cycle analysis indicated that DLX2 was highly expressed during transition from G1 phase to S phase. Thereafter, it was confirmed that the cyclin A and CDK2 expression levels were obviously increased in the progression of cell-cycle. In addition, how exogenous DLX2 affected the biologic behaviors of EOC cells was investigated. The protein expression levels of CDK2 and PCNA were decreased through shDLX2 down-regulation of DLX2 expression. Effect of DLX2 on apoptosis of EOC cells transfected with DLX2-shRNA or control-shRNA was detected to determine the effect of DLX2 on the progression of EOCs. The flow cytometry analysis indicated that the apoptosis rate of DLX2-shRNA-transfected EOC cells was higher than that in the control group. This study also indicated that knocking down DLX2 expression increased the apoptosis rate of EOC cells. However, more studies are needed to investigate the specific effect of DLX2 on the progression of EOCs.

To sum up, our study confirms the effect of DLX2 on cellular proliferation of EOCs, and it is indicated that knocking down DLX2 expression induces cell cycle deregulation and has a close association with the occurrence of EOCs. DLX2 may serve as a new therapeutic or preventative target for EOCs. It is very meaningful for resolving the debate on the role of DLX2 in progression of EOCs as a prognosis indicator. Therefore, it is necessary to clarify the role of DLX2 in EOC progression in the future studies.

Author contributions

MHH, YLZ and YH designed the research study. QHX performed the research. YFJ and YWH provided help and advice on the cell and tissue experiments. YLL analyzed the data. MHH, HLK and QZ wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Nantong University (approval number: 0196 (2016)).
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Conflict of interest

The authors declare no conflict of interest.

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