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Significance of differential expression of OLFM4 in the development of endometrial adenocarcinoma

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Abstract

The incidence of endometrial adenocarcinoma (EA) has increased worldwide in recent years due to the widespread use of estrogen therapy and the overall increase in life expectancy. However, we know of no sensitive molecular index that can be used to predict the onset of EA, evaluate the therapeutic effects of treatment agents, or provide prognostic benefit in post-treatment follow-up. To explore the correlation between human olfactomedin 4 (OLFM4) and the clinicopathologic parameters of EA, and to determine the precise involvement of OLFM4 as a related factor in the occurrence and development of EA. We enrolled 61 gynecologic patients for a retrospective study at the Tai'an Central Hospital of Shandong Province from January 1, 2016, to June 30, 2022. We determined the expression levels of estrogen receptor α (ERα), progesterone receptor (PR), and OLFM4 proteins in endometrial tissue with the immunohistochemical S-P staining method, and analyzed the correlations among ERα, PR, and OLFM4 protein expression levels and with the pathologic stage, histologic grade, myometrial invasiveness, and lymphatic metastasis of EA. The expression levels of OLFM4 in EA were higher than in normal endometrium (P = .036). The expression level of OLFM4 protein in stage II-III patients was higher than that in stage I patients (P = .034), and the expression levels of ERα and PR proteins in EA were lower than those in normal endometrial tissue (P = .014 and P = .0005). While we observed no correlation in endometrial tissues of disparate pathologic types between OLFM4 and the expression levels of ERα and PR proteins, we noted a positive correlation between the expression levels of ERα and PR protein. The expression level of OLFM4 protein increased with the malignant degree of endometrial lesions and OLFM4 protein expression was related to the FIGO stage of EA. And OLFM4 protein can be used as 1 of the potential diagnostic factors for endometrial lesions, which is worthy of further study.

Abbreviations: AEH = atypical endometrial hyperplasia, EA = endometrial adenocarcinoma, EC = endometrial carcinoma, ERα = estrogen receptor α, OD = optical density, OLFM4 = human olfactomedin 4, PR = progesterone receptor.

Keywords: endometrial adenocarcinoma, ERα, immunohistochemistry, OLFM4, PR

1. Introduction

Endometrial carcinoma (EC) is a malignant tumor that originates from the endometrial glands, and the most recent statistical data show that endometrial adenocarcinoma (EA) ranks first among women's malignant reproductive tract tumors and that its incidence is rising.[1] Bokhman first proposed 2 types of EC types in 1983 – estrogen-dependent and non-estrogen-dependent EC[2] – with EA constituting the estrogen-dependent EC type. EA is also known as type I EC, is related to continuous stimulation by estrogen, exhibits a high incidence rate, and accounts for 75% of patients with EC. Non-endometrioid adenocarcinoma (i.e., estrogen-independent EC) is known as type II EC and principally includes endometrial serous carcinoma as uterine serous carcinoma and clear cell carcinoma, which show incidence rates of 5 % to 10% and 1% to 5%, respectively. Non-endometrioid adenocarcinoma is primarily noted in postmenopausal women; does not relate to estrogen, tamoxifen, or other stimuli; and its prognosis is poor.[3] The treatment of EC depends upon the surgical pathologic stage and high-risk factors of recurrence that enable an appropriate surgical plan and auxiliary measures to be chosen.

The authors has no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The early stage mainly comprises surgical treatment, and late-stage patients are treated surgically and/or with radiotherapy and/or drugs (e.g., chemotherapeutic agents and hormones).[4] Although the incidence of EA is high, the currently employed tumor markers manifest low specificity and sensitivity in the evaluation of therapeutic effects and follow-up after treatment.[5,6]

Human olfactomedin 4 (OLFM4, also known as GW112 protein and hGC-1) is a member of the olfactory mediator protein family that contains the "olfacto-medin" domain. OLFM4 is widely expressed in the human stomach, small intestine, colon, bone marrow, prostate, pancreas, and other normal tissues.[7] OLFM4 protein is known to be involved in the onset and development of inflammatory and immune diseases such as in inflammatory mucosa with ulcerative colitis[8] and rheumatoid arthritis[9]; in gastric biopsy tissue infected by Helicobacter pylori and in other inflammatory diseases[10]; as well as in malignant tumor diseases such as lung cancer,[11] breast cancer,[12] prostate cancer, gastric cancer,[13,14] esophageal cancer,[15] pancreatic cancer,[16] liver cancer,[17] and gallbladder cancer.[18] The expression of OLFM4 in cervical squamous cell carcinoma[19,20] tissues is elevated relative to that in healthy normal tissues; and the expression levels of OLFM4 in highly differentiated gastric cancer, colon cancer, prostate cancer, breast cancer,[12] and cervical squamous cell carcinoma tissues are significantly higher than in poorly differentiated cancer tissues.[12,21,22] Intriguingly, OLFM4 protein expression levels appear to reflect inconsistencies among different diseases within the unified physiologic systems of the body. For example, the expression level of OLFM4 protein varies among disparate types of myeloid leukemia, with augmented expression in M4 but no abnormal expression in M1, M2, or M5 myeloid leukemias.[23] Although OLFM4 gene mRNA was found to be expressed in the early stages of colon cancer, breast cancer, and lung cancer, its presence was not related to the expression of apoptotic molecules, indicating that it could be used independently in the diagnosis of these 3 tumors. OLFM4 is additionally a protective factor in the occurrence of ovarian cancer and is downregulated by the microRNA mir-486-5p, contributing to the tumorigenesis of ovarian cancer.[24] In contradistinction, estrogen-receptor signaling downregulates mir-486-5p and upregulates the expression of OLFM4, retarding the development and progression of ovarian cancer.[24]

Whether OLFM4 is related to the onset and development of EA is currently less frequently studied, both in China and internationally. In the present study, we therefore applied a retrospective methodology to investigate and determine OLFM4, estrogen receptor α (ERα), and progesterone receptor (PR) proteins in tumor samples taken from a variety of patients, elucidate the correlation of these molecules with clinicopathologic parameters of EA, and explore the relationships of OLFM4 with endometrial diseases and EA. Our work exhibits important clinical reference value in guiding the treatment of EA that includes gene therapy, prognostic evaluation, and improvement in our understanding of the biologic behaviors of malignant tumors.

2. Clinical data

A total of 61 endometrial tissue samples were obtained from gynecologic surgery at the Department of Pathology of Tai'an Central Hospital from January 1, 2016, to June 30, 2022. We then divided the patients into the following groups: 18 patients in the normal endometrial group (including 9 in the proliferative stage and 9 in the postmenopausal stage) aged between 47 and 61 years, with an average age of 49.1 years; 9 patients with atypical endometrial hyperplasia (AEH) aged 42 to 64 years, with an average age of 49.7 years; and 34 patients with EA aged 46 to 55 years, with an average age of 51.4 years. With respect to the pathologic staging of FIGO surgery in 2009, there were 16 patients in stage I, 10 patients in stage II, and 8 patients in stage III. We noted 7 patients with positive lymph node metastasis; 27 with negative lymph node metastasis; 14 with high, medium, or low differentiation (i.e., G1, G2, and G3); 17 with less than 1/2 myometrial invasion depth; and 17 patients with over 1/2 myometrial invasion depth.

Our inclusion criteria were no exposures to radiotherapy, chemotherapy, or hormonal treatment before the operation; and we observed no other complications. Patients in the proliferative-endometrium group underwent total hysterectomy due to the benign diseases of hysteromyoma or adenomyosis; and postoperative pathology confirmed that these individuals were in proliferative or secretory phases of their menstrual cycles. The patients in the group with AEH were treated in the outpatient department, underwent diagnostic curettage or total hysterectomy at the hospital, and postoperative pathology confirmed AEH. Patients in the EA group were diagnosed with EA upon curettage in our outpatient department due to abnormal uterine bleeding and were hospitalized for surgery and pathologic staging. Permission from the ethics committee of Tai'an central hospital was passed for his survey study.

2.1. Experimental and statistical methods

We evaluated OLFM4, ERα, and PR protein expression levels in various types of endometrial tissues using the immunohistochemical SP staining method, and calculated the mean optical density (OD).[25] We applied Microsoft Excel 2007 to establish our database and GraphPad Prism 9 software to analyze and plot the data, and adopted independent sample *t* tests to analyze OLFM4 and ERα protein levels. The relationship between PR and different clinicopathologic features was examined with Spearman's rank correlation analysis.

2.2. Determination of experimental results

OLFM4 protein was principally expressed in the cytoplasm, and a positive reaction was reflected as brownish yellow. ERα protein was primarily localized to the nucleus and cytoplasm of endometrial gland cells, and exhibited a brownish yellow or brown coloration. Cells expressing PR protein were chiefly located in the nucleus and cytoplasm, and also showed a brownish-yellow or brown color. We calculated the mean OD for each observational index within each slice, randomly selected 3 visual fields from each film, and used AIpathwell software to assess the cumulative OD values and positively pixelated areas. The mean OD value was then calculated as the cumulative OD value/positively pixelated area.[26]

3. Results

3.1. Expression of ERα, PR, and OLFM4 proteins in normal endometrium, AHE, and EA is depicted in Figure 1A–I

We herein implemented immunohistochemical staining to detect the levels of OLFM4 protein in proliferative endometrium, AEH, and EA; and compared their mean ODs. The mean OLFM4 protein OD in proliferative endometrium was significantly lower than that in EA ($P = .036$, Fig. 1K) but did not differ from postmenopausal endometrium and atypical proliferative endometrium ($P > .05$, Fig. 1J and K). Although the mean OD for OLFM4 protein in AEH appeared lower than that in EA, this was not statistically significant ($P > .05$, Fig. 1K). The rise in the mean OD of OLFM4 protein was concomitant with increasing pathologic grade ($R = 0.374$, $P = .004$), and its expression level increased with aggravation of the disease.

The mean OD of ERα protein in the AEH group showed a tendency to be reduced compared with that in the hyperplastic-endometrium group but not to a statistical degree ($P > .05$). However, the mean OD for ERα protein in EA was lower than

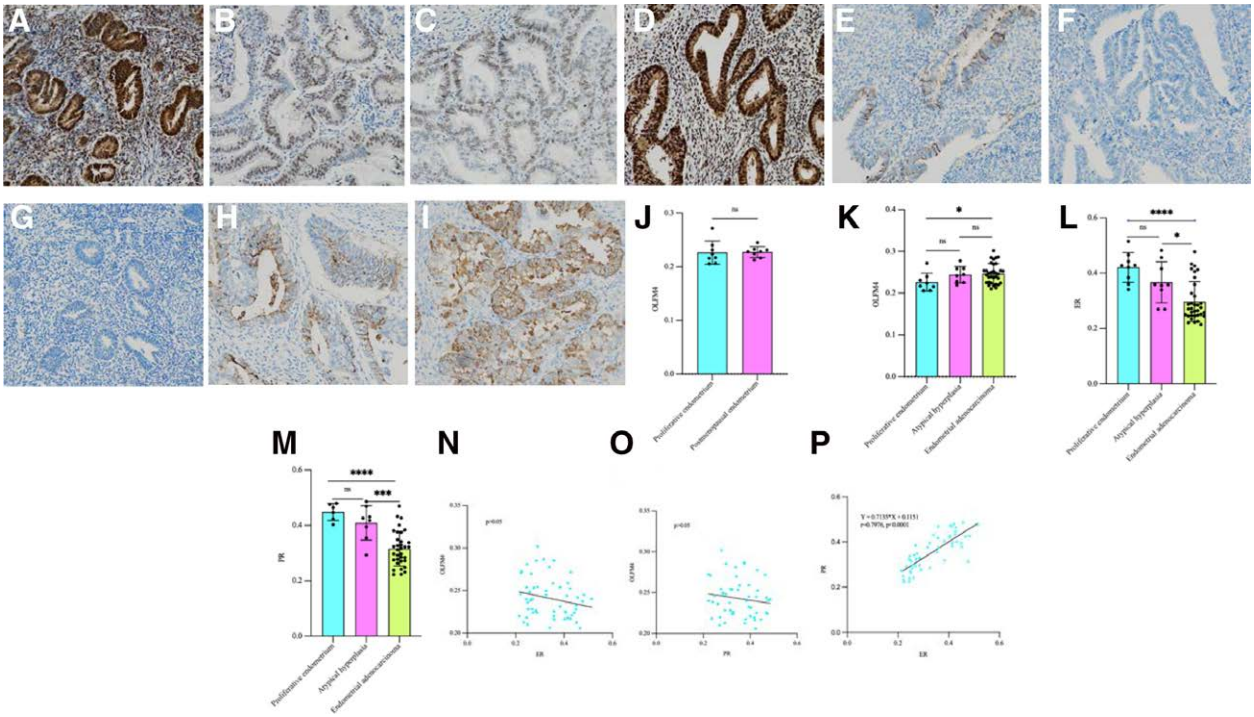

Figure 1. IHC staining shows the expression of OLFM4, ERα, and PR proteins in various types of endometrial tissues. (A–C) ERα protein expression in endometrial tissue of proliferative endometrium, AEH, and EA. (D–F) PR protein expression in endometrial tissue of proliferative endometrium, AEH, and EA. (G–I) OLFM4 protein expression in endometrial tissue of proliferative endometrium, AEH, and EA. (J–M) Expression of OLFM4, ERα, and PR in proliferative endometrial tissue, AEH tissue, and EA tissue. (N) Correlation between OLFM4 and ERα protein expression in proliferative endometrial tissue, AEH tissue, and EA tissue. (O) Correlation between OLFM4 and PR protein expression in proliferative endometrial tissue, atypical proliferative endometrial tissue, and EA tissue. (P) Correlation between ERα and PR protein expression in proliferative endometrial tissue, atypical proliferative endometrial tissue, and EA tissue. AEH = atypical endometrial hyperplasia, EA = endometrial adenocarcinoma, EC = endometrial carcinoma, ERα = estrogen receptor α, IHC = immunohistochemical, OLFM4 = human olfactomedin 4, PR = progesterone receptor.

that in the AEH group ($P = .014$). Additionally, the mean OD for ERα protein in the EA group was attenuated relative to the proliferative endometrium group ($P < .0001$, Fig. 1L). The expression level of ERα protein was negatively correlated with the degree of endometrial lesion ($r = –0.589$, $P = .000$) and fell concomitantly with aggravation of the lesion.

The mean OD for PR protein in the AEH group was statistically reduced compared with that in the hyperplastic group ($P > .05$), as was the PR protein OD in the EA group relative to the AEH group ($P = .0005$). The positive expression for PR protein in the EA was also significantly lower than that in the proliferative endometrium group ($P < .0001$, Fig. 1M). In addition, the positive expression for PR protein was negatively correlated with the severity of the lesion ($r = –0.603$, $P = .000$), with its expression level diminished with aggravation of the lesion.

3.2. The relationship between the positive expression of OLFM4, ERα, PR, and the clinicopathologic features of EA (Fig. 2)

The mean OD for OLFM4 protein in patients with stage I EA differed from that in patients with stage II-III EA ($P = .034$, Fig. 2A). However, we noted no significant difference in the positive expression rate for OLFM4 protein between individuals with a muscle-layer infiltration depth < 1/2 and those with an infiltration depth ≥ 1/2 ($P > .05$, Fig. 2D). There was likewise neither a difference in the mean OD for OLFM4 protein among G1, G2, and G3 groups ($P > .05$, Fig. 2G); nor in the ODs between EA patients with lymph node metastasis and those without metastasis ($P > .05$, Fig. 2J).

We observed no significant differences in the mean OD for ERα protein-positive patients with a muscle-layer infiltration depth < 1/2 versus ≥ 1/2 ($P = .032$, Fig. 2E), or for ERα positivity between patients with stage I EA vs. stage II-III EA ($P > .05$, Fig. 2B). ER protein-positivity neither differed among G1, G2, and G3 groups ($P > .05$, Fig. 2H); nor between patients with EA and lymph node metastasis and patients without lymph node metastasis ($P > .05$, Fig. 2K).

PR protein also did not vary between patients with stage I EA and those exhibiting stage II-III EA ($P > .05$, Fig. 2C); among G1, G2, and G3 groups ($P > .05$, Fig. 2I); between patients showing muscle-layer infiltration depth < 1/2 and ≥ 1/2 ($P > .05$, Fig. 2F); or between EA patients with lymph node metastasis and those without lymph node metastasis ($P > .05$, Fig. 2L).

3.3. Correlation analysis of OLFM4, ERα, and PR protein expression in EA

Of tissues collected from the 61 cases in the proliferative-endometrium, AEH, and EA groups, we noted no correlations between OLFM4 and the mean ODs for ERα or PR proteins ($P > .05$, Fig. 1N and O), while the mean ODs for ERα and PR proteins were positively correlated with each other ($R = 0.7976$, Fig. 1P). Similarly, in 34 cases of EA, OLFM4 was not correlated with mean ERα and PR protein ODs ($P > .05$, Fig. 2M and N), but mean ODs were positively correlated between ERα and PR proteins ($R = 0.6966$, Fig. 2O).

4. Discussion

The results of the present study revealed that OLFM4 protein expression levels in EA tissue were higher than that in normal proliferative endometrium, and that the expression levels in phase II-III patients were higher than those in phase I

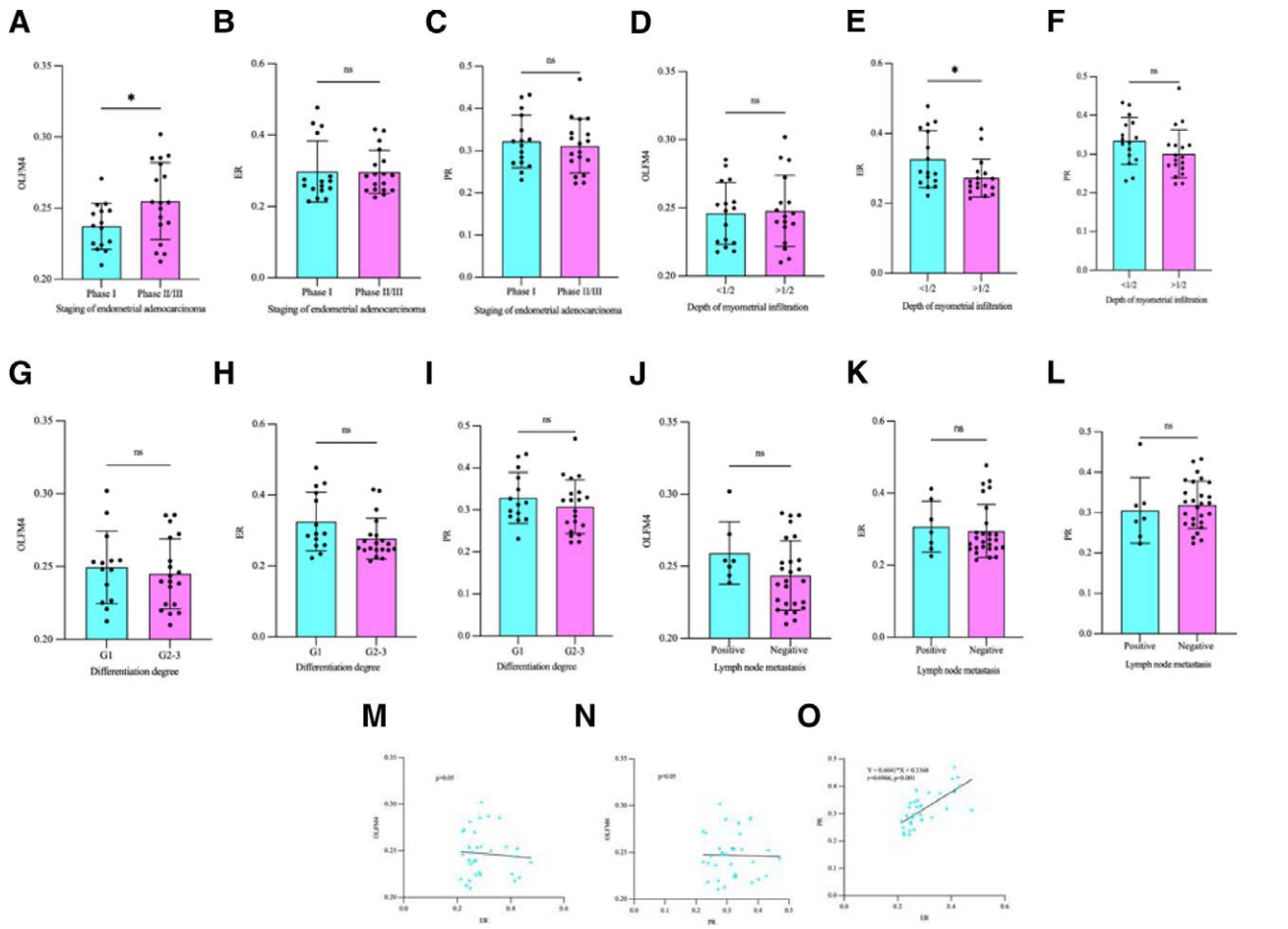

Figure 2. The expression of OLFM4, ERα, and PR proteins in EA with respect to diverse pathologic parameters and the correlations among their expression levels. (A–2) Expression of OLFM4, ERα, and PR proteins in endometrial tissues of EA at various stages. (D–F) Expression of OLFM4, ERα, and PR proteins in EA manifesting > 1/2 or < 1/2 myometrial invasion depth. (G–I) Expression of OLFM4, ERα, and PR proteins in endometrial tissues with G1 and G2-3 differentiation in EA. (J–L) Expression of OLFM4, ERα, and PR proteins in EA lymph node-metastasis negative and positive endometrial tissues. (M) Correlation between OLFM4 and ERα protein expression in EA. (N) Correlation between protein expression levels of OLFM4 and PR in EA. (O) Correlation between the expression of ERα protein and PR protein in EA. EA = endometrial adenocarcinoma, ERα = estrogen receptor α, OLFM4 = human olfactomedin 4, PR = progesterone receptor.

patients – indicating that OLFM4 protein expression showed a commensurate increase with aggravation of endometrial tissue lesions consistent with the data of Duan et al. These authors conducted PCR analysis of normal endometrium, AEH, and EA, and demonstrated that OLFM4 mRNA showed the same increasing trend as in the above tissues.[27] This study also depicted OLFM4 expression levels as related to surgical and pathologic stages. The expression level of OLFM4 in stage II-III patients was significantly elevated relative to that in stage I patients; that is, the higher the stage, the higher the expression level of OLFM4. However, OLFM4 protein expression level was not related to the depth of myometrial invasion, lymph node metastasis, or the degree of differentiation of EA. It was confirmed for other systemic diseases that the positive expression level of OLFM4 protein in well-differentiated gastric cancer, colon cancer, prostate cancer, and cervical squamous cell carcinoma was significantly higher compared with poorly differentiated cancer.[12,21,22] OLFM4 expression is thus a potential predictor of lymph node metastasis in early gastric cancer. The combination of OLFM4 with tumor size and differentiation also allowed improved stratification of early gastric cancer patients with differential risks for lymph node metastasis.[14] The reduced expression of OLFM4 in gastric cancer was related to lymph nodes, distant metastasis, and poor prognosis; and provided a reference for further study of the application of OLFM4 to the diagnosis of endometrial diseases. However, the results of this study and those of other scholars show that OLFM4 undoubtedly plays a role in the early development of EA. In the current study, the positive expression levels for ERα and PR proteins in normal proliferative endometrial tissue, atypical proliferative endometrial tissue, and EA tissue gradually diminished, congruent with the studies by other investigators.[28] The expression of ERα and PR protein was positively correlated in our patients' proliferative endometrium, atypical proliferative endometrium, and EA; while the expression of OLFM4 protein was not associated with the expression of ERα and PR proteins, indicating that OLFM4 was not essentially involved in the onset and development of EA via ERα- and PR-mediated cellular-signaling pathways.

OLFM4 protein was highly expressed in some cancers and precipitated the development of patients' diseases, playing an anti-cancer role, while basic research revealed that OLFM4 protein exerted an anti-apoptotic function.[8] During the onset and development of cervical and gallbladder cancers, OLFM4 protein acts as a tumor suppressor and inhibits cervical cancer metastasis by regulating mTOR signaling[19]; and OLFM4 deletion enhances the sensitivity of gallbladder cancer cells to cisplatin.[29]

There are few extant studies on OLFM4 action in uterine diseases. Compared with the endometrium of normal healthy

women, OLFM4 mRNA levels of endometriotic endometrium were augmented, suggesting that OLFM4 is likely to be critical to the onset and development of endometriosis[24] and providing a participatory role for OLFM4 in the occurrence of endometrial diseases. In addition, OLFM4 is also involved in the development of other diseases of the female reproductive system. For example, the expression level of OLFM4 protein in ERα-positive breast cancer was significantly correlated with cancer stage, distant metastasis, and specific survival of breast cancer patients. In ER-negative breast cancer, however, OLFM4 protein was not associated with distant metastasis or prognosis. Therefore, OLFM4 appears to be related to an invasive phenotype of ER-positive breast cancer and acts as an effective marker of distant metastasis in ER-positive breast cancer patients.[19] OLFM4 is the gene most closely associated with stem-cell-like cell clusters,[30] which can then initiate cellular growth and differentiative abilities in primary organoids. These activities are necessary for the effective growth of primary organoids and comprise an important rationale for the commensurate increase in OLFM4 expression with augmented invasiveness of malignant tumors. As HIF-1α participates in the aforementioned process, some scholars have proposed that the OLFM4/HIF-1α axis constitutes a target signaling pathway in the development of novel drugs for the treatment of HCC.[31] The super enhancer-related long noncoding RNA ac005592.2 promotes tumor progression by positively regulating OLFM4 in colorectal cancer,[32] and OLFM4 is crucial to the onset of digestive system diseases. OLFM4 is highly expressed in polymorphonuclear bone marrow-derived inhibitory cells in colitis and colorectal cancer, its expression levels and cell populations are positively correlated with the progression of inflammatory bowel disease toward colon cancer, and it displays resistance to PD1 treatment in colon cancer.[33]

The augmented expression of OLFM4 protein in some diseases engenders a more unfavorable prognosis, indicating that it occupies a role in promoting cancer.[7] A study of its molecular mechanism showed that the fusion of the OLFM4 gene and the protooncogene RET constitutes a carcinogenic factor in tumorigenesis.[16] Under hypoxia, knockdown of OLFM4 enhances the sensitivity of A549 cells to cisplatin, and knockdown of OLFM4 expression attenuates the invasive capability of A549 cells provoked by hypoxia.[34] The cancer-promoting effect of OLFM4 is induced by the Lgr5/Wnt-signaling pathway, which is closely related to invasive tumor progression and poor prognosis in liver cancer by regulating the proliferation of tumor cells and tumor stem cell-like characteristics induced by Stat3; and patients with elevated OLFM4 expression manifest a high incidence of hematologic recurrence. Multivariate analysis showed that high OLFM4 expression was an independent factor in poor prognosis. We therefore postulate OLFM4 protein as a new predictor and potential therapeutic target for predicting the prognosis of patients with liver cancer.[17]

In conclusion, the expression level of OLFM4 protein increased with the malignant degree of endometrial lesions. Although OLFM4 protein expression was related to the FIGO stage of EA, it was not associated with the depth of myometrial invasion, lymph node metastasis, or cancer tissue differentiation. We also did not observe any correlation between OLFM4 and the expression levels of ER and PR protein in degenerative endometrial diseases. Therefore, the results of this study indicate that OLFM4 plays a role in endometrial diseases not through the signaling pathway mediated by ER and PR receptors, and that it is thus worthy of further investigation.

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