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Upregulation of h-TERT and Ki-67 in ectopic endometrium is associated with recurrence of endometriosis

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At present, endometriosis remains a worldwide health burden, with the main symptoms of dysmenorrhea, chronic pelvic pain, and infertility, markedly reducing the quality of life (de Ziegler et al., 2010). Although there is no proof that the disease is associated with high mortality, this disorder can significantly contribute to the deterioration of women's general well-being (McPeak et al., 2018). The main current treatment for endometriosis is surgery to remove endometriotic lesions; however, the recurrence rate following surgical treatment is as high as 21.5% at two years and 40.0%–50.0% at five years post-surgery (Koga et al., 2015). To prevent recurrence, adjuvant treatment with drugs after surgery is recommended to prolong relapse-free intervals. However, it is inconvenient for patients to continuously use such medications in terms of adverse effects and cost (Turk, 2002).

In this respect, it is highly important to carefully select patients that are predicted to benefit the most from medication therapy. Thus far, the causes of recurrence are not fully known, and no biomarkers with prognostic significance have been found. A large number of epidemiologic studies focusing on risk factors for the recurrence of endometriosis have been published, and many scholars aimed to find prognostic molecular markers, while their findings were often conflicting (Bozdag, 2015; Ceccaroni et al., 2019; Li XY et al., 2019; Liu et al., 2020).

To date, little attention has been paid to the identification of available biomarkers during the perioperative period or the prediction of recurrence. The availability of these biomarkers and the risk factors for recurrence may accordingly identify patients with high recurrence risk, prompting intervention to postpone or even prevent a possible recurrence.

In this study, the expression profile and clinical significance of human telomerase reverse transcriptase (h-TERT) and Ki-67 in recurrent endometriosis were investigated. We further evaluated the association between h-TERT level in the ectopic endometrium and the postoperative serum carbohydrate antigen 125 (CA125) level respectively and endometriosis recurrence, in order to help explore the pathophysiological mechanisms of recurrence and identify high-risk patients.

In the present study, a total of 30 ovarian endometrioma patients with recurrence, 30 ovarian endometrioma patients without recurrence, and 30 controls with endometrium tissue samples were compared. The recurrent ovarian endometriosis group consisted of patients with recurrent ovarian endometrioma 30 months after a previous surgery, the non-recurrent ovarian endometriosis group consisted of patients without recurrent ovarian endometrioma 30 months after a previous surgery, and the control group included myoma patients undergoing laparoscopy with matched basal characteristics. The clinical characteristics of patients in the three groups are shown in Table 1. No differences were observed in age, body mass index (BMI), employment status, marital status, place of

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Table 1 Clinical characteristics of all participants

Characteristics	All (n=90)	Recurrent ovarian endometriosis group (n=30)	Non-recurrent ovarian endometriosis group (n=30)	Control patients (n=30)	P value
Age (years)	38.8±6.8	36.9±6.8	35.2±5.3	42.7±4.1	<0.001
BMI (kg/m^2)	21.9±5.7	20.3±4.8	22.1±5.8	21.6±5.5	0.563
Employment status					0.786
Unemployed or retired	18 (20.0)	6 (20.0)	8 (26.7)	4 (13.3)	
Part-time	12 (13.3)	4 (13.3)	3 (10.0)	5 (16.7)	
Full-time	60 (66.7)	20 (66.7)	19 (63.3)	21 (70.0)	
Marital status					0.177
Married or cohabiting	17 (18.9)	6 (20.0)	4 (6.7)	7 (23.3)	
Unmarried, divorced, or widowed	73 (81.1)	24 (80.0)	26 (86.7)	23 (76.7)	
Place of residence					0.620
Urban	52 (57.8)	18 (60.0)	16 (53.3)	18 (60.0)	
Suburban	18 (20.0)	7 (23.3)	6 (20.0)	5 (16.7)	
Rural	20 (22.2)	5 (16.7)	8 (26.7)	7 (23.3)	
Level of education					0.328
Primary or below	12 (13.3)	4 (13.3)	2 (6.7)	6 (20.0)	
Secondary	31 (34.4)	12 (40.0)	10 (33.3)	9 (30.0)	
College or beyond	47 (52.2)	14 (46.7)	18 (60.0)	15 (50.0)	
Gravidity	1 (1−2)	1 (1−2)	1 (1−2)	1 (1−3)	0.987
Parity	1 (1−2)	1 (1−1)	1 (1−2)	1 (1−2)	0.786
Abortion	1 (1−2)	1 (1−2)	1 (1−2)	1 (1−2)	0.902
Dysmenorrhea					0.018
None	33 (36.7)	6 (20.0)	7 (23.3)	20 (66.7)	
Mild	23 (25.6)	9 (30.0)	10 (33.3)	4 (13.3)	
Moderate	21 (23.3)	8 (26.7)	9 (30.0)	4 (13.3)	
Severe	13 (14.4)	7 (23.3)	4 (13.3)	2 (6.7)	
Menstrual cycle phase					0.123
Proliferative	79 (87.8)	27 (90.0)	26 (86.7)	26 (86.7)	
Secretory	11 (12.2)	3 (10.0)	4 (13.3)	4 (13.3)	
r-AFS score		46.3±5.7	44.6±6.2		0.534

Data are expressed as mean±standard deviation (SD), median (interquartile range), or number (percentage). BMI: body mass index; r-AFS: revised American Fertility Society.

residence, level of education, gravidity, parity, abortion, or revised American Fertility Society (r-AFS) score among the three groups. However, patients with endometriosis had a higher prevalence of severe dysmenorrhea.

Patients with endometriosis exhibited obviously increased levels of serum CA125 compared with controls. Furthermore, the levels of postoperative serum CA125 were significantly higher in the recurrent ovarian endometriosis group, as compared with those in the non-recurrent ovarian endometriosis group (Fig. 1).

The expression levels of h-TERT and Ki-67 protein were detectable by immunohistochemistry in eutopic or ectopic endometrium on tissue blocks from patients in the three study groups. A higher incidence of h-TERT and Ki-67 expression was observed in ectopic endometrium in the endometriosis groups, as compared with that of eutopic endometrium in the control group (Fig. 2). As for patients with recurrent ovarian endometrioma, the incidences of h-TERT and Ki-67 expression were significantly higher compared with the non-recurrent ovarian endometrioma patients (Fig. 2).

As described above, higher serum levels of CA125 and higher endometrial h-TERT levels were found in patients with recurrent ovarian endometrioma, as compared with patients without the recurrence of ovarian endometrioma. Whether there is a correlation between serum CA125 and endometrial h-TERT expression is still to be investigated. The correlation analysis

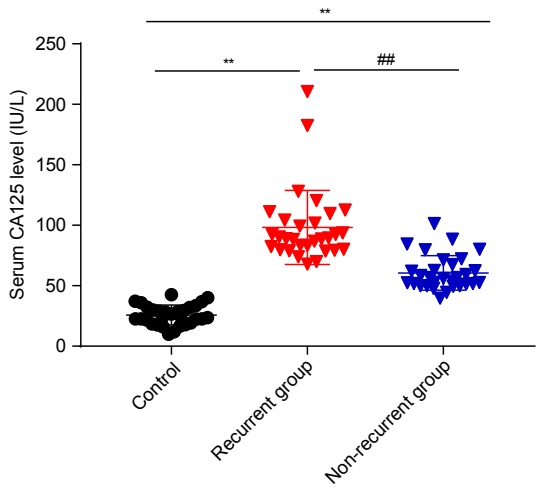

Fig. 1 Postoperative serum levels of CA125 in subjects from all three groups. Each point indicates the value of CA125 concentration in the serum of an individual patient; the bar shows the standard error of mean in each group. ** *P*<0.01, vs. the control group; ## *P*<0.01, vs. the non-recurrent group. Control: patients without endometriosis; Recurrent group: endometrioma patients with recurrence within 30 months after a previous surgery; Non-recurrent group: endometrioma patients without recurrence within 30 months after a previous surgery. CA125: carbohydrate antigen 125.

revealed that h-TERT protein expression in ectopic endometrium was positively associated with the serum CA125 levels of patients with ovarian endometrioma (Fig. 3).

Endometriosis, a common and challenging health issue of reproductive-aged women, comes at a high cost to individuals and society (Chapron et al., 2019). After decades of research, it is still difficult to develop new drug therapies and treatments for endometriosis due to too many unexplained molecular dissimilarities between endometriosis lesions and eutopic endometrium (Rolla, 2019). Furthermore, conflicts still exist for some of these molecular dissimilarities. For now, surgery remains the gold standard both for definitive diagnosis and an effective method for the treatment of endometriomas (Greene et al., 2016). However, the risks of potential decreases in ovarian reserve and recurrence should be balanced carefully (Chiang et al., 2015; Hanege et al., 2019). Recurrences are common and often rapid in patients at high risk. However, the high-risk factors for relapse are still not clear, resulting in difficulties in making precise postoperative treatment plans for affected patients.

Although endometriosis is not a neoplastic disease, it still features certain processes indicating metastasis and carcinogenesis, including cell motility, adhesion, invasion, angiogenesis, and metaplasia (Vercellini et al., 2014; Laganà et al., 2019; Li and Wang, 2021). Telomerase activity is mainly determined by the h-TERT enzyme, the catalytic and rate-limiting component of telomerase activity (Poole et al., 2001). Telomerase expression is specifically correlated with cell proliferation (Hu et al., 2017; Hannen and Bartsch, 2018), and most endometrial cancers express high levels of telomerase (Alnafakh et al., 2019). Considering the complex cellular and molecular mechanisms involved in the progression of endometriosis, and the similarities between endometriosis and tumorigenesis and metastasis (Kajiyama et al., 2019; Kalaitzopoulos et al., 2020), we assumed a possible relationship between telomerase and endometriosis development. Li Y et al. (2019) reported that intrafollicular tumor necrosis factor-α (TNF-α) might down-regulate h-TERT via the nuclear factor-κB (NF-κB) pathway in granulosa cells from endometriosis patients. However, whether h-TERT dysregulation in ectopic endometrium is associated with the development or recurrence of endometrioma needs to be investigated.

As mentioned above, endometriosis shares certain features of tumorigenesis. There has been growing interest in the role of Ki-67 monoclonal antibody in endometriosis development. Ki-67 is a nuclear protein associated with cellular proliferation (Miller et al., 2018). Although little is known about the specific roles of Ki-67, it is present in all active phases of the cell cycle but absent in resting (G0) cells (Miller et al., 2018). The expression level of Ki-67 protein is determined as a labeling index (LI) of tissue specimens. Nowadays, Ki-67 LI is used to predict the prognosis, survival, and even the recurrence of tumors (Bubendorf et al., 1996). In a study by Park et al. (2009), endometrial cell proliferation in patients with endometriosis was found to be higher than that in patients without endometriosis, as determined by the Ki-67 proliferation index. A higher level of Ki-67 LI is associated with aggressive tumoral behavior and metastasis (Li et al., 2015). Whether Ki-67 is linked to the recurrence of endometrioma is still unknown. In the present study, we presumed that h-TRET and Ki-67 may be related to the recurrence of endometrioma, as shown by immunohistochemistry through the higher expression indices of h-TRET and Ki-67 in the ectopic endometrium of patients with recurrent endometrioma, as

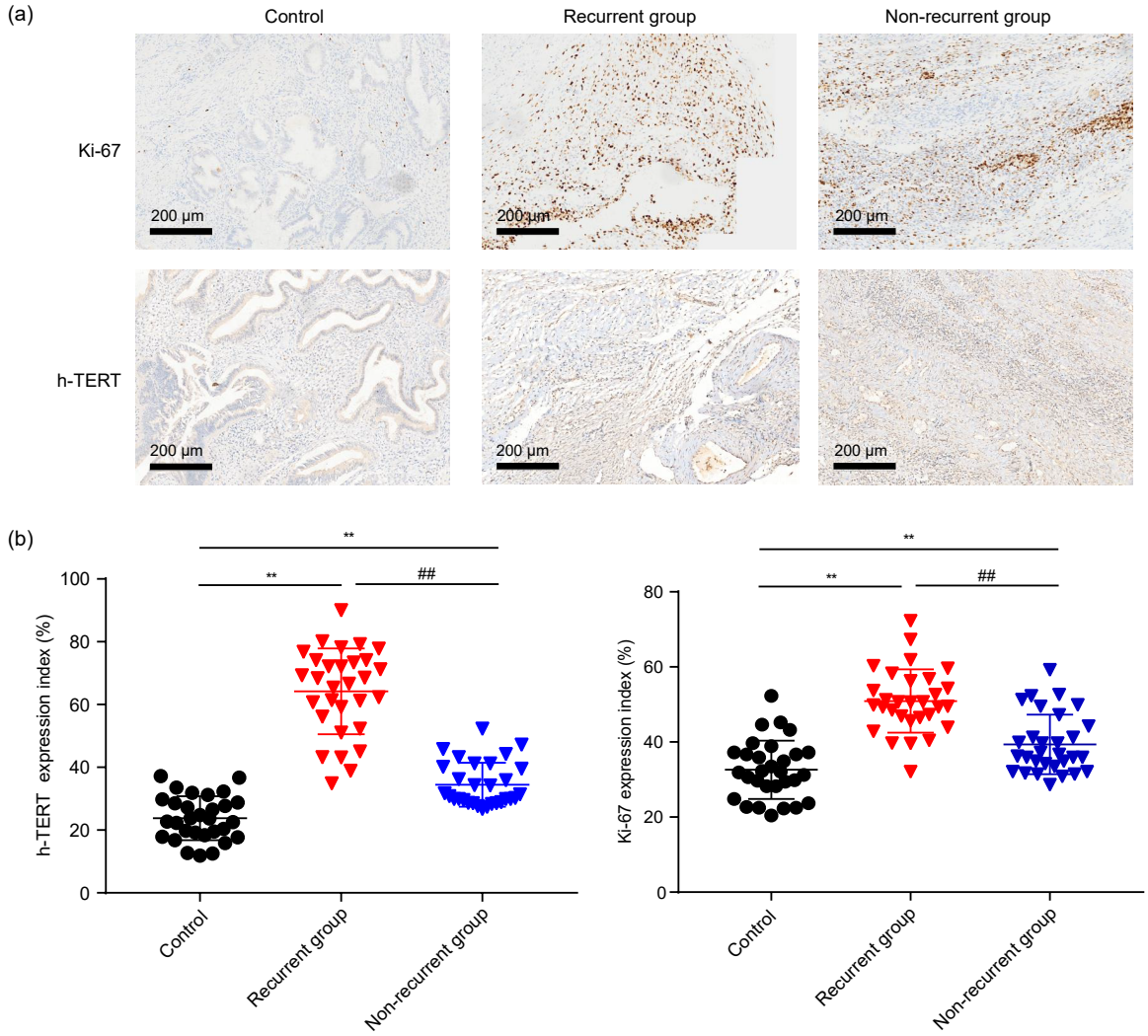

Fig. 2 Expression of h-TERT and Ki-67 in the eutopic endometrium of subjects in the control group and in the ectopic endometrium of subjects with endometrioma in the recurrent and non-recurrent groups, as determined by immunohistochemistry (a) and the corresponding quantitative analysis (b). Each point in (b) indicates the expression index of h-TERT or Ki-67 in the endometrium of each patient; the bar shows the standard error of mean in each group. ** $P<0.01$ vs. the control group; $^{\#\#}$ $P<0.01$ vs. the non-recurrent group. Control: patients without endometriosis; Recurrent group: endometrioma patients with recurrence within 30 months after a previous surgery; Non-recurrent group: endometrioma patients without recurrence within 30 months after a previous surgery. h-TERT: human telomerase reverse transcriptase.

compared with patients without the recurrence of endometrioma.

CA125, a glycoprotein produced by coelomic epithelial tissues such as the peritoneum, has been the most extensively studied biomarker for endometriosis (Socolov et al., 2017). Although it is clinically used as a marker for the diagnosis of endometriosis or therapeutic effect evaluation, its association with endometriosis development is still controversial. In the present study, we found that the postoperative serum CA125 level was higher in patients with recurrent endometrioma, which offers a possibility for CA125 to act as a predictive marker for the recurrence of endometriosis. However, we only included 30 patients in this study; therefore, further studies with larger sample size are needed to confirm this association and to determine the appropriate prediction threshold. We further found that serum CA125 levels were positively associated with the expression index of h-TRET in the ectopic endometrium of endometriosis patients. Meanwhile, whether higher levels of CA125 may lead to the upregulation of h-TRET in the endometrium, or the

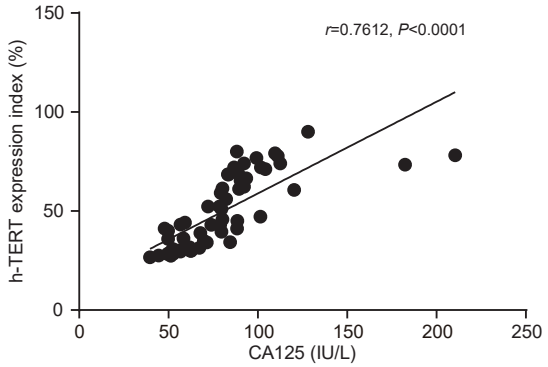

Fig. 3 Distribution of the h-TERT expression index according to the serum levels of CA125 in endometriosis patients. The linear regression line indicates associations between the serum levels of CA125 and h-TERT expression in the ectopic endometrium of endometriosis patients with endometrioma (n=60, r=0.7612, P<0.0001). h-TERT: human telomerase reverse transcriptase; CA125: carbohydrate antigen 125.

upregulation of h-TRET in endometriosis could result in elevated CA125 production by the endometrium, is yet to be investigated. It has been reported that inflammation might downregulate the expression of h-TERT through the activation of NF-κB (Sakamoto et al., 2003). Elevated serum CA125 levels can be observed in patients with chronic inflammation (Ding et al., 2020). We assumed that there might be an association among inflammation, NF-κB signaling, and serum CA125 level. Therefore, the serum CA125 level may be correlated with h-TRET expression in the endometrium via NF-κB signaling; further in vivo and in vitro studies are necessary to verify this assumption.

In conclusion, the present study demonstrated that the upregulation of h-TERT and Ki-67 in ectopic endometrium is associated with the recurrence of endometriosis. The serum CA125 level may affect the recurrence of endometriosis via the regulation of h-TERT, while further research is needed to explore the mechanisms involved in the association between serum CA125 and endometrial h-TERT expression. Our study provided potential predictive markers for the recurrence of endometriomas and shed light on the mechanism involved, thus contributing to more precise postoperative treatment for this life changing illness.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

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Author contributions

Jie LUO, Jun LIN, and Jianhong ZHOU conceived the study, participated in its design and coordination, and helped to draft the manuscript. Zhimin SONG carried out the immunohistochemistry studies and drafted the manuscript. Tao ZHANG and Ketan CHU were involved in clinical data collection and data analysis, and Jingyi LI was involved in revising the manuscript. All authors have read and approved the final manuscript, and therefore, take responsibility for the integrity of the study.

Compliance with ethics guidelines

Jie LUO, Zhimin SONG, Tao ZHANG, Ketan CHU, Jingyi LI, Jianhong ZHOU, and Jun LIN declared that they have no conflict of interests.

The study was approved by the Human Ethics Committee of Women's Hospital, Zhejiang University School of Medicine (No. 20190014), China. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study. Additional informed consent was obtained from all patients for whom identifying information is included in this article.

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Supplementary information

Materials and methods