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Molecular expression characteristics confirm the malignancy concealed by morphological alterations in endometrial cancer after fertility-preserving treatment

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Abstract

Purpose Fertility-preserving treatment (FPT) has been widely used for young patients with early stage endometrial cancer (EC). However, the literature on the effectiveness and safety of FPT remains controversial. The aim of this study was to investigate malignant transformation in EC after FPT by immunohistochemistry (IHC).

Methods A retrospective analysis of pre- and post-treatment biopsy specimens from 24 patients with grade 1 endometrioid adenocarcinoma (EAC) or complex atypical hyperplasia (CAH) was performed. The expression levels of ARID1A, PTEN, and β-catenin were assessed by IHC.

Results The protein expression levels of ARID1A, PTEN, and β-catenin were not significantly different between pre- and post-treatment specimens. However, there was a significant difference between pre-treatment and normal specimens as well as between post-treatment and normal specimens. The protein expression of β-catenin was significantly increased in patients with progression compared with those without progression after FPT.

Conclusion The morphologic normalization of patients with EC after FPT may not be accompanied by the absence of tumor malignancy, and β-catenin may serve as a biomarker for the response to FPT. These results may contribute to a better understanding of the malignant transformation of EC after FPT and the optimization of treatment strategies for young patients with birth plans.

Keywords Endometrial cancer · Fertility-preserving treatment · Molecular expression · Malignant change

Introduction

Endometrial cancer (EC) is the most common cancer of the female genital tract, accounting for 3.6% of all new cancer cases and 1.8% of all cancer deaths in America in 2017 [1]. Although EC is typically experienced by postmenopausal women, 5–10% of women diagnosed with EC are under the age of 40 [2]. Complex atypical hyperplasia (CAH) is thought to be a precursor of EC; approximately, 25% of cases with CAH will progress to EC if left untreated [3]. The current standard of treatment for patients diagnosed with grade 1 EC is total hysterectomy with bilateral salpingo-oophorectomy. However, for patients at reproductive age with the desire to preserve fertility, especially nulliparous patients, fertility-preserving treatment (FPT) may be considered [4–7]. According to the guidelines of the National Comprehensive Cancer Network (NCCN) and European Society of Gynecological Oncology (ESGO), FPT should be restricted to patients with FIGO stage IA, well-differentiated endometrioid adenocarcinoma (EAC), and no evidence of myometrial invasion [8, 9]. Although FPT for EC has been widely used in recent years, there is a lack of agreement between studies on the effectiveness and safety of FPT [10, 11]. There is still the risk of disease progression and recurrence

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even though the uterine morphology shows a normal endometrium (NE) after FPT. A meta-analysis study revealed that FPT for EC could achieve a pooled regression rate of 76.2% and a relapse rate of 40.6% [12].

Various studies have demonstrated that the progression of EC is accompanied by various molecular changes [13–15]. For example, the loss of phosphatase and tensin homolog (PTEN), a tumor suppressor that negatively regulates the PI3K-AKT signaling pathway, is greater in women with EC compared with those with benign endometrial hyperplasia or an NE [13, 16]. A study has reported that cytoplasmic PTEN protein loss was significantly higher in endometrioid tumors (75%) than in non-endometrioid tumors (43%) [17]. AT-rich interactive domain-containing protein 1 A (ARID1A) is a recently identified tumor suppressor that is involved in the regulation of cellular differentiation, tissue development, and DNA repair through the SWI/SNF complex [18]. The nuclear loss of ARID1A has been detected in 19–44% cases of EC [18, 19]. Several studies have demonstrated that β-catenin, a protein encoded by the CTNNB1 gene, plays a critical role in cell–cell adhesion and is a constituent of the Wnt pathway [20]. The nuclear accumulation of β-catenin is a characteristic of EC [21, 22]. Saegusa et al. reported that 29% of grade 1 EC cases had a positive nuclear expression of β-catenin; in contrast, no expression was detected in the NE [22].

The objective of this study was to examine molecular changes in sequential biopsies obtained before and after FPT from 24 patients diagnosed with grade 1 EC or CAH through immunohistochemistry (IHC). The results could shed light on the malignant changes concealed by morphological alterations in these patients.

Materials and methods

Patient selection

This study was approved by the Institutional Ethics Committee of West China Second University Hospital. Between 2008 and 2016, 24 patients with grade 1 EAC and CAH underwent FPT; pre- and post-treatment formalin-fixed, paraffin-embedded tissues were retrospectively identified (Fig. 1). The sample set included 17 patients with grade 1 EAC and seven patients with CAH, and 24 individuals with an NE in the proliferative phase served as the control group. At the time of diagnosis, all pathology slices from the endometrial biopsy were assessed by two experienced pathologists specializing in gynecologic oncology at our institution. The diagnostic criteria for CAH included the presence of nuclei that are stratified with the loss of polarity, nuclei that are enlarged and rounded with irregular shapes, nuclei with the coarsening of chromatin creating a vesicular appearance, nuclei with prominent nucleoli, nuclei with mitotic activity (variable), a cytoplasm with eosinophilia (diffuse or focal), and a markedly increased gland/stroma ratio. The diagnostic criteria for grade 1 EAC included a confluent glandular pattern in which individual glands (uninterrupted by the stroma) merge and create a cribriform pattern, an

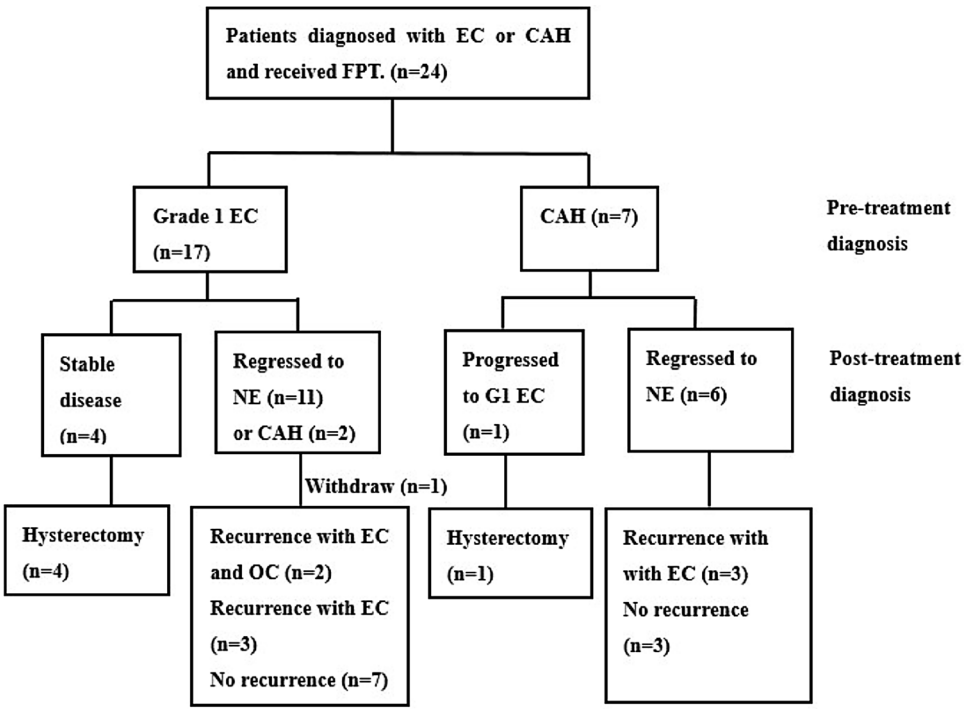


Fig. 1 Study design. *EC* endometrial cancer, *CAH* complex atypical hyperplasia, *NE* normal endometrium, *OC* ovarian cancer, *G1* well differentiated

extensive papillary pattern, and an irregular infiltration of glands associated with an altered fibroblastic stroma (desmoplastic response) in accordance with the 1994 World Health Organization (WHO) classification. All the biopsies were available for repeated sampling, and the last retrieved biopsy specimens were used to evaluate the therapeutic effect after FPT. Disease regression was defined as changes from EC to CAH/NE or from CAH to NE. Disease progression was defined when there was no evidence of disease regression. Recurrence was defined as the presence of EC or CAH during follow-up after an endometrial sample indicated an NE at the end of FPT. Disagreements between the pathologists were resolved by consensus.

IHC and evaluation

IHC for PTEN, ARID1A, and β-catenin was performed as described previously [22, 23]. EC, CAH, and NE paraffin sections (4 μm) were de-waxed in xylene and ethanol, rehydrated in phosphate-buffered saline (PBS), and treated for 10 min with 3% hydrogen peroxide in methanol to block endogenous peroxidase activity. Antigen retrieval was performed by incubating the sections in 10 mM citric acid (pH 6.0) at 121 °C for 15 min. The sections were blocked with 10% normal goat serum and incubated overnight at 4 °C with anti-PTEN antibody (1:100; Novusbio, Centennial, CO, USA), anti-ARID1A antibody (1:1000; Abcam, Cambridge, MA, USA), and anti-β-catenin antibody (1:500; Abcam, Cambridge, MA, USA). After washing with PBS, the sections were incubated with biotinylated rabbit anti-goat immunoglobulin antibody (ZSGB-Bio Ltd., Beijing, China), and the reaction products were visualized by staining with diaminobenzidine (Dako Ltd., Glostrup, Denmark) followed by counterstaining with hematoxylin.

The slides were scored independently by two investigators (L.L. and T.H.) without knowledge of the clinical data. The immunoreactivity was scored semi-quantitatively by considering the percentage and intensity of the stained tumor cells as previously described [24, 25]. The histological score (H-score) was obtained using the following formula:

$$\begin{aligned}\text{H-score} = {} & 1 \times (\%\ \text{light staining}) \\ & + 2 \times (\%\ \text{moderate staining}) \\ & + 3 \times (\%\ \text{strong staining}).\end{aligned}$$

In all cases, NE or stromal cells served as the internal positive control. Disagreements were resolved by consensus.

Statistical analysis

The statistical significance of IHC results was evaluated by paired *t* test. All statistical analyses were performed using SPSS version 21.0.

Results

Clinical characteristics of patients

A total of 24 patients with initial biopsy results indicating EC (17) or CAH (7) were enrolled; the study design is outlined in Fig. 1. The mean age at diagnosis was 29.3 ± 3.6 years, and the mean body mass index (BMI) was 22.4 ± 3.7. They were treated with medications including megestrol acetate, medroxyprogesterone acetate, goserelin acetate, dydrogesterone, and levonorgestrel. The treatment duration ranged from 3 to 24 months with a mean of 6.5 ± 4.6 months. The follow-up was conducted using biopsy specimens, and the duration ranged from 8 months to 81 months with a mean of 38.8 ± 26.7 months. After FPT, 19 patients (79.2%) showed disease regression, of whom eight patients (44.4%) relapsed during follow-up in this study. The other 11 patients with remission attempted to conceive; seven patients (63.6%) achieved pregnancy, and five patients (45.5%) gave birth to a healthy child. The characteristics of all 24 patients are summarized in Table 1.

Protein expression changes in patients after FPT

IHC analysis of ARID1A, PTEN, and β-catenin antibodies was performed for all specimens, and the results were evaluated using the H-score (Fig. 2). For ARID1A and β-catenin, their nuclear expression was regarded as positive. For PTEN, its cytoplasmic expression was regarded as positive. Paired *t* test showed that there were no significant differences between pre- and post-treatment specimens in the expression levels of ARID1A, PTEN, and β-catenin ($p = 0.053$, 0.503, and 0.434, respectively). Moreover, the expression levels of ARID1A and PTEN in NE specimens were significantly higher than in pre- and post-treatment specimens with p values of < 0.01. The expression level of β-catenin was markedly decreased in NE specimens compared with pre- and post-treatment specimens ($p = 0.038$ and 0.028, respectively). The H-score results of IHC analysis are shown in Fig. 3a–c.

Association of protein expression with disease progression and disease recurrence

To investigate the potential of ARID1A, PTEN, and β-catenin in predicting disease progression or recurrence,

Table 1 Patient characteristics

Patients	Age at diagnosis	BMI	Pre-diagnosis GxPx	Initial biopsy result	Treatment, mg/day	Treatment duration, months	Final biopsy result	Follow-up, months	Recurrence	Hysterectomy	GxPx at the end of follow-up
1	27	22.1	G0P0	EC	MA, 160	6	Normal	26	No	No	G1P1
2	27	21.6	G0P0	EC	MA, 160	6	CH	32	No	No	G1P0
3	29	19.1	G1P0	EC	MA, 160	3	Normal	9	No	No	G1P0
4	23	20.7	G0P0	EC	MA, 160	6	Normal	83	Yes	Yes	G0P0
5	28	18.3	G0P0	EC	MA, 160	3	EC	8	TF	Yes	G0P0
6	25	27	GOPO	EC	MA, 160	3	CAH	9	No	No	GOPO
7	26	27.1	G0P0	EC	MA, 160	3	Normal	26	Yes	Yes	G0P0
8	33	23.3	G0P0	CAH	MA, 160 + GA, 3.6 g/mo	5 + 6	Normal	8	No	No	G0P0
9	28	19	G0P0	CAH	MPA, 250 + LNG-IUS	18 + 6	CH	31	Yes	Yes	G0P0
10	31	28	G1P1	EC	LNG-IUS	6	CAH	9	No	No	G1P1
11	28	30.8	G0P0	EC	MA, 160 + LNG-IUS	6 + 5	EC	10	TF	Yes	G0P0
12	22	30.4	G0P0	EC	MA, 160 + LNG-IUS	3 + 3	EC	22	TF	Yes	G0P0
13	30	20.2	G0P0	EC	MPA, 250	3	EC	34	TF	Yes	G0P0
14	29	18.4	G1P0	EC	MA, 160	3	Normal	36	Yes	Yes	G1P0
15	29	22.9	G0P0	EC	MA, 160	7	Normal	31	No	No	G1P1
16	29	24	G0P0	CAH	MA, 160	10	Normal	43	No	No	G1P1
17	29	21	G1P0	EC	MA, 160	8	Normal	48	Yes	Yes	G2P1
18	29	17.6	G0P0	CAH	MA, 160 + MPA, 250	6 + 3	EC	69	TF	Yes	G0P0
19	35	19.8	G0P0	CAH	MA, 160 + DG, 20	3 + 5	Normal	73	Yes	Yes	G0P0
20	35	20.4	G2P1	EC	MA, 160	3	Normal	72	Yes	Yes	G3P1
21	37	21.5	G1P0	EC	MA, 160	3	Normal	72	Withdraw		G1P0
22	31	21.9	G0P0	EC	MA, 160 + MPA, 250	3 + 3	Normal	80	No	No	G0P0
23	33	20.1	G0P0	CAH	MA, 160	3	Normal	81	Yes	No	G0P0
24	30	23.4	G0P0	CAH	MA, 160	6	Normal	18	No	No	G1P1

BMI body mass index, *G* gravida, *P* para, *EC* endometrial cancer, *CAH* complex atypical hyperplasia, *MA* megestrol acetate, *GA* goserelin acetate, *MPA* medroxyprogesterone acetate, *LNG-IUS* Levonorgestrel-releasing intrauterine system, *DG* dydrogesterone, *CH* complex hyperplasia, *TF* treatment failure

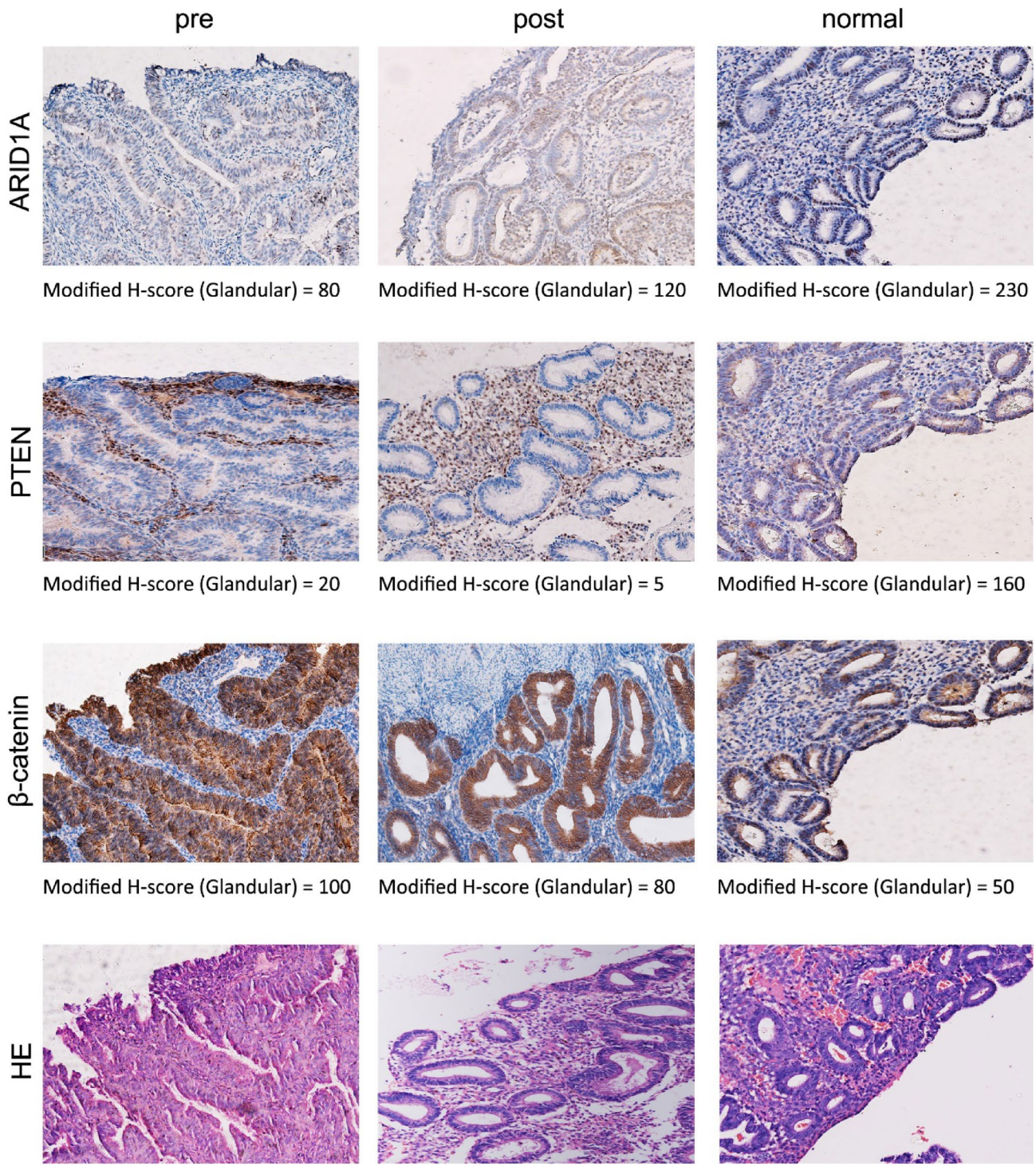


Fig. 2 IHC results of pre-FPT, post-FPT, and NE biopsy specimens. The nuclear expression of ARID1A and β-catenin and the cytoplasmic expression of PTEN were regarded as positive (×200). HE: hematoxylin–eosin staining

all patients were divided into the "progression" and "no progression" groups as well as the "recurrence" and "no recurrence" groups. IHC results revealed no substantial differences between the expression levels of the three proteins and disease progression before FPT (Fig. 4a). However, the nuclear expression level of β-catenin was significantly increased in the "progression" group compared with the "no progression" group ($p = 0.028$) after FPT (Fig. 4b, e, f). The expression levels of ARID1A and PTEN were not significantly associated with disease

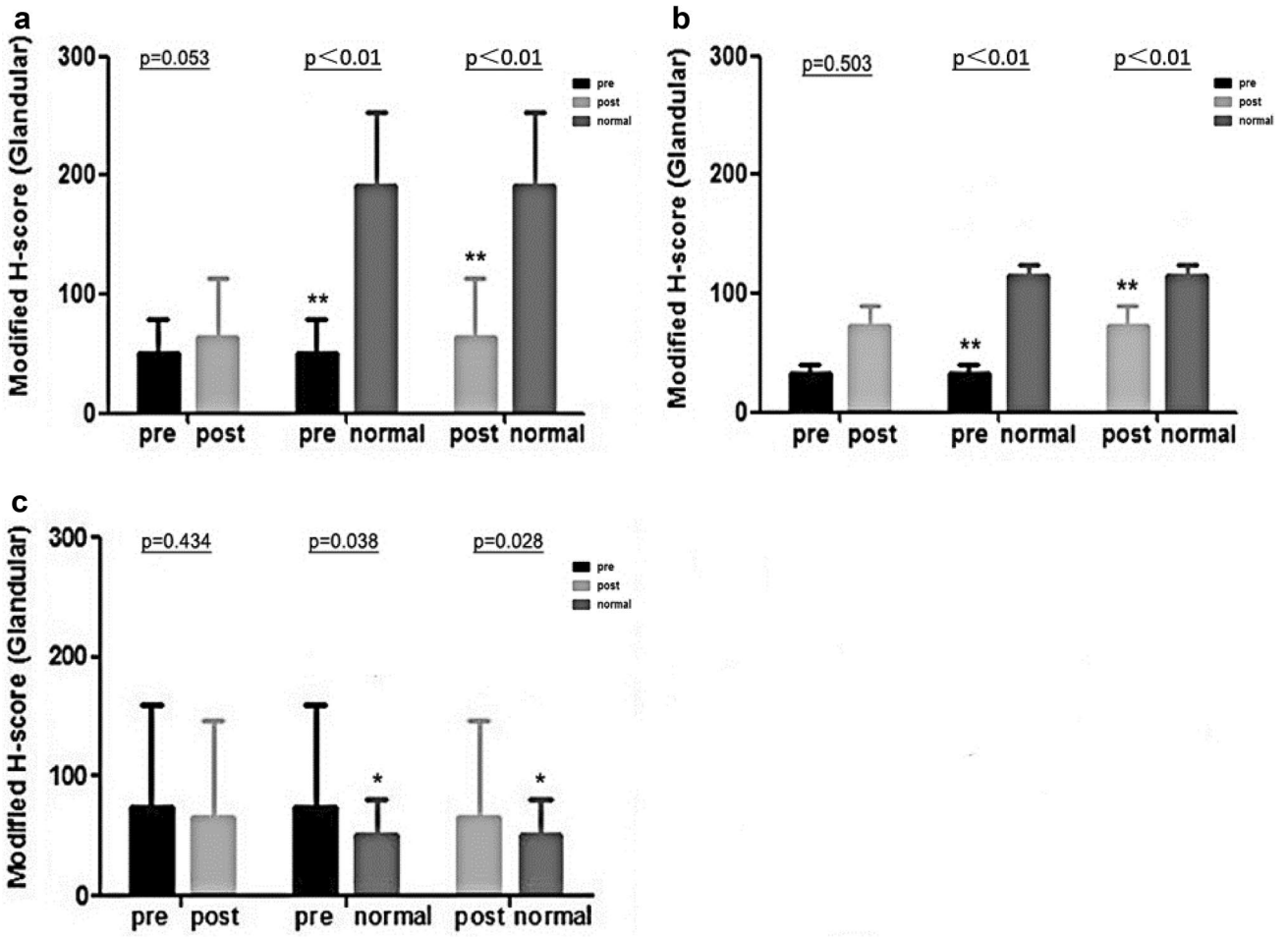


Fig. 3 Effect of FPT on molecular expression levels in biopsy specimens. Average modified *H*-score values of **a** ARID1A, **b** PTEN, and **c** β-catenin in pre-FPT, post-FPT, and NE biopsy specimens. *$p < 0.05$; **$p < 0.01$

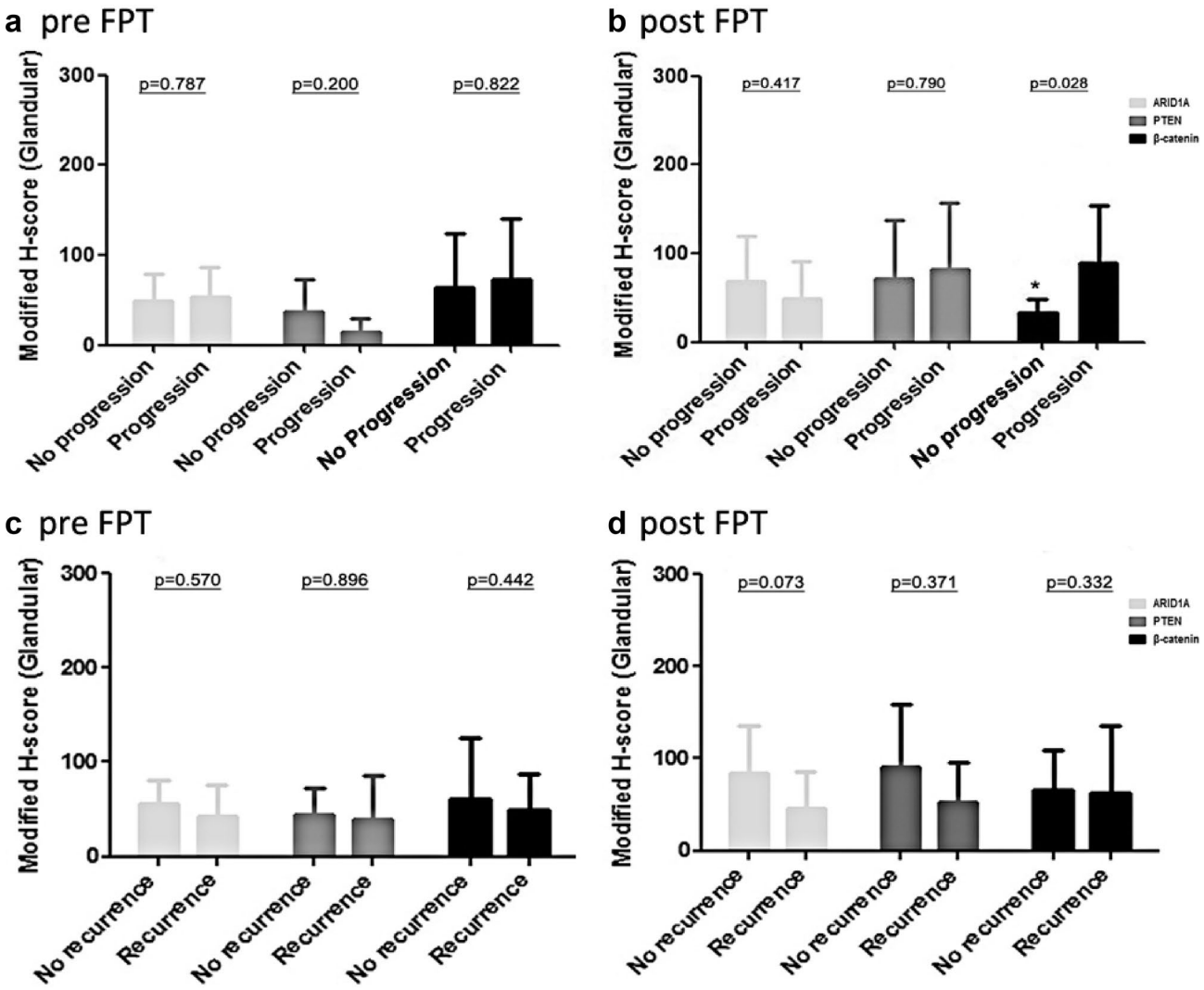
progression after FPT (Fig. 4b). Moreover, there was no significant association between the expression levels of the three proteins and disease recurrence before or after FPT (Fig. 4c, d).

Discussion

The objective of this study was to determine changes in tumor malignancy in patients with early stage EC or CAH who underwent FPT by analyzing the expression levels of three proteins (ARID1A, PTEN, and β-catenin), which have been reported to be involved in endometrial tumorigenesis [13, 14]. Overall, the IHC analysis of biopsy specimens revealed significant differences in the expression levels of ARID1A, PTEN, and β-catenin between pre- and post-treatment samples. These results suggest that the malignancy of EC was still maintained even though morphologic diagnosis indicated a normal status after FPT. In addition, the high expression of β-catenin after FPT may be a sign of treatment failure.

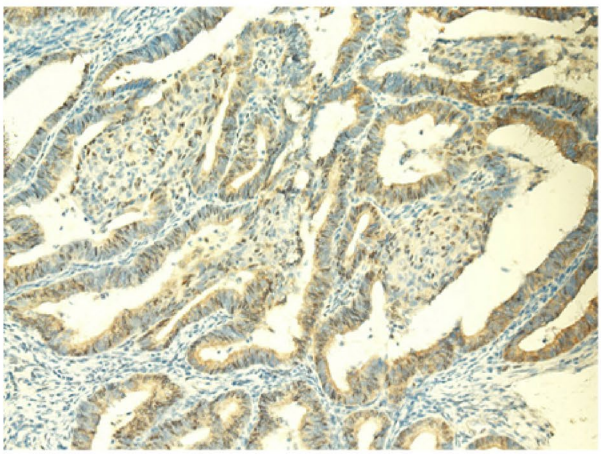
The remaining EC malignancy after FPT may be attributed to several reasons. According to the guidelines of the NCCN [8], the gold standard for the diagnosis of EC is the dilatation and curettage (D&C) method, which is mostly based on morphologic evaluation. A study by Visser et al. [26] indicated that the pooled agreement rate between preoperative endometrial sampling and the final diagnosis for tumor grade was 0.67 (95% CI 0.60–0.75), and the agreement between hysteroscopic biopsy and the final

Fig. 4 Molecular expression of endometrial biopsy specimens (progression vs. no progression and recurrence vs. no recurrence). **a**, **b** Average modified H-score values of patients with or without progression (pre-FPT and post-FPT). **c**, **d** Average modified H-score values of patients with or without recurrence (pre-FPT and post-FPT). **e**, **f** Representative IHC images of the β-catenin staining of endometrial biopsy specimens from patients with progression and regression after FPT (×200). *$p < 0.05$

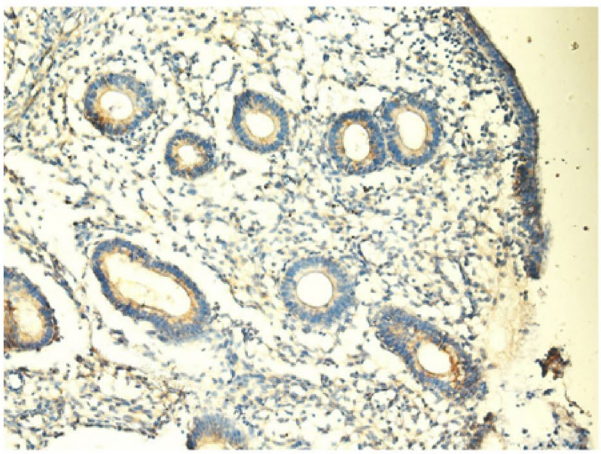


e progression after FPT

f no progression after FPT



Modified H-score (Glandular) =120



Modified H-score (Glandular) =10

diagnosis was higher than for D&C ($p = 0.02$). Therefore, the diagnostic accuracy of D&C is somehow limited. On the other hand, progesterone treatment is based on the concept that unopposed estrogen stimulation is the driver for both the initiation and progression of EC [27]. However, not all patients have a good response to progestin. The mechanism of progesterone resistance is not fully understood. Progestin receptor (PR) downregulation resulting from continuous progestin administration could lead to desensitization to progestin, which was thought to be one of the reasons for progestin resistance. In addition, other molecules including those in the EGF/EGFR and insulin signaling pathways may contribute to progestin resistance [28, 29]. The results of our study showing malignancy retention after FPT may explain the high recurrence rate in around half (54%) of the patients with CAH or grade 1 EC who initially responded to treatment [30].

Recently, there has been increasing interest in identifying potential biomarkers to predict the response to progesterone therapy in patients with EC or CAH. In a previous study, a positive PR status was identified to correlate with treatment responsiveness; however, only approximately 75% of these patients responded to progesterone treatment, indicating that other prognostic biomarkers are required for this subset of patients [31]. Another study reported that the mRNA expression of FOXO1 was significantly decreased by 28.9-fold after conservative therapy in the "progression" group vs. the "no progression" group [32]. A study by van Gent et al. indicated that the Wnt or PI3K/Akt status could not predict the response to progesterone treatment in patients with EC [33], which is inconsistent with our results showing that the increased nuclear expression of β-catenin after FPT was significantly associated with disease progression. Considering the different patient ethnicities, IHC evaluation methods, and sample size, the analytical performance of β-catenin needs to be investigated more extensively.

This study investigated malignant changes based on the molecular expression of ARID1A, PTEN, and β-catenin after progesterone treatment in patients with EC or CAH. In addition, the association of the expression patterns of the three proteins with disease progression and recurrence was determined. The results of our study revealed that the morphological normalization of the endometrium after progesterone therapy may not be accompanied by the absence of tumor malignancy, which is consistent with the high recurrence rate after FPT in patients with EC. More importantly, these results demonstrated that hysterectomy with bilateral salpingo-oophorectomy should be considered when patients completed childbearing or changed their mind on wanting a child, as recommended in the guidelines of the NCCN or ESGO [8, 9]. Further studies with a focus on elucidating the mechanism of malignant transformation in patients with EC who underwent FPT would be interesting.

Nevertheless, this study has some limitations. Although we analyzed the pre- and post-treatment biopsies of a cohort of patients, the number of samples included was relatively small. Moreover, the proteins we assessed were restricted to ARID1A, PTEN, and β-catenin. In addition, the patients included in the study did not receive a uniform treatment or follow-up regimen. It is possible that the patients who were sampled already had EC even though the initial sampling results showed a benign pathology. However, this can only be confirmed by hysterectomy.

In conclusion, the present study revealed that there was no statistical difference in the expression levels of ARID1A, PTEN, and β-catenin in the biopsy specimens of patients with EC or CAH before and after FPT. Furthermore, the expression of β-catenin was significantly increased in patients who progressed compared with those who did not. Therefore, the absence of morphological abnormalities in patients with EC after FPT may not be accompanied by the absence of tumor malignancy. This finding is of importance in understanding the malignant transformation of EC after FPT and optimizing treatment strategies for patients with EC who want to preserve fertility.

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Author contributions ZyL: Hypothesis and project development; TwyH: Data collection, specimen collection, data analysis, and manuscript drafting; LL: Data analysis; EY: Data collection and specimen collection; DN: IHC and RT-PCR.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the Institutional Ethics Committee of West China Second University Hospital. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent As this was a retrospective study, formal consent was not required. However, informed consent was obtained from each patient for specimen collection.

References

1. Siegel RL, Miller KD, Jemal A (2017) Cancer statistics. CA Cancer J Clin 67(1):7–30

2. Gallup DG, Stock RJ (1984) Adenocarcinoma of the endometrium in women 40 years of age or younger. Obstet Gynecol 64(3):417–420
3. Kurman RJ, Kaminski PF, Norris HJ (1985) The behavior of endometrial hyperplasia A long-term study of "untreated" hyperplasia in 170 patients. Cancer 56(2):403–412
4. Montz FJ, Bristow RE, Bovicelli A, Tomacruz R, Kurman RJ (2002) Intrauterine progesterone treatment of early endometrial cancer. Am J Obstet Gynecol 186(4):651–657
5. Gotlieb WH, Beiner ME, Shalmon B, Korach Y, Segal Y, Zmira N, Koupolovic J, Ben-Baruch G (2003) Outcome of fertility-sparing treatment with progestins in young patients with endometrial cancer. Obstet Gynecol 102(4):718–725
6. Jobo T, Imai M, Kawaguchi M, Kenmochi M, Kuramoto H (2000) Successful conservative treatment of endometrial carcinoma permitting subsequent pregnancy: report of two cases. Eur J Gynaecol Oncol 21(2):119–122
7. Kaku T, Yoshikawa H, Tsuda H, Sakamoto A, Fukunaga M, Kuwabara Y, Hataeg M, Kodama S, Kuzuya K, Sato S, Nishimura T, Hiura M, Nakano H, Iwasaka T, Miyazaki K, Kamura T (2001) Conservative therapy for adenocarcinoma and atypical endometrial hyperplasia of the endometrium in young women: central pathologic review and treatment outcome. Cancer Lett 167(1):39–48
8. Koh WJ, Abu-Rustum NR, Bean S, Bradley K, Campos SM, Cho KR, Chon HS, Chu C, Cohn D, Crispens MA, Damast S, Dorigo O, Eifel PJ, Fisher CM, Frederick P, Gaffney DK, George S, Han E, Higgins S, Huh WK, Lurain JR, Mariani A, Mutch D, Nagel C, Nekhlyudov L, Fader AN, Remmenga SW, Reynolds RK, Tillmanns T, Ueda S, Wyse E, Yashar CM, McMillian NR, Scavone JL (2018) Uterine neoplasms, version 1.2018, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 16(2):170–199
9. Rodolakis A, Biliatis I, Morice P, Reed N, Mangler M, Kesic V, Denschlag D (2015) European Society of Gynecological Oncology Task Force for fertility preservation. Int J Gynecol Cancer 25(7):1258–1265
10. Baek JS, Lee WH, Kang WD, Kim SM (2016) Fertility-preserving treatment in complex atypical hyperplasia and early endometrial cancer in young women with oral progestin: is it effective? Obstet Gynecol Sci 59(1):24–31
11. Carneiro MM, Lamaita RM, Ferreira MC, Silva-Filho AL (2016) Fertility-preservation in endometrial cancer: is it safe? Review of the literature. JBRA Assist Reprod 20(4):232–239
12. Gallos ID, Yap J, Rajkhowa M, Luesley DM, Coomarasamy A, Gupta JK (2012) Regression, relapse, and live birth rates with fertility-sparing therapy for endometrial cancer and atypical complex endometrial hyperplasia: a systematic review and metaanalysis. Am J Obstet Gynecol 207(4):261–266
13. Sanderson PA, Critchley HO, Williams AR, Arends MJ, Saunders PT (2017) New concepts for an old problem: the diagnosis of endometrial hyperplasia. Hum Reprod Update 23(2):232–254
14. Murali R, Soslow RA, Weigelt B (2014) Classification of endometrial carcinoma: more than two types. Lancet Oncol 15(7):e268–e278
15. DeSouza LV, Grigull J, Ghanny S, Dube V, Romaschin AD, Colgan TJ, Siu KW (2007) Endometrial carcinoma biomarker discovery and verification using differentially tagged clinical samples with multidimensional liquid chromatography and tandem mass spectrometry. Mol Cell Proteomics 6(7):1170–1182
16. Baak JP, Van Diermen B, Steinbakk A, Janssen E, Skaland I, Mutter GL, Fiane B, Lovslett K (2005) Lack of PTEN expression in endometrial intraepithelial neoplasia is correlated with cancer progression. Hum Pathol 36(5):555–561
17. Djordjevic B, Hennessy BT, Li J, Barkoh BA, Luthra R, Mills GB, Broaddus RR (2012) Clinical assessment of PTEN loss in endometrial carcinoma: immunohistochemistry outperforms gene sequencing. Mod Pathol 25(5):699–708
18. Werner HM, Berg A, Wik E, Birkeland E, Krakstad C, Kusonmano K, Petersen K, Kalland KH, Oyan AM, Akslen LA, Trovik J, Salvesen HB (2013) ARID1A loss is prevalent in endometrial hyperplasia with atypia and low-grade endometrioid carcinomas. Mod Pathol 26(3):428–434
19. Mao TL, Ardighieri L, Ayhan A, Kuo KT, Wu CH, Wang TL, Shih I (2013) Loss of ARID1A expression correlates with stages of tumor progression in uterine endometrioid carcinoma. Am J Surg Pathol 37(9):1342–1348
20. Aberle H, Butz S, Stappert J, Weissig H, Kemler R, Hoschuetzky H (1994) Assembly of the cadherin-catenin complex in vitro with recombinant proteins. J Cell Sci 107(Pt 12):3655–3663
21. Scholten AN, Creutzberg CL, van den Broek LJ, Noordijk EM, Smit VT (2003) Nuclear β-catenin is a molecular feature of type I endometrial carcinoma. J Pathol 201(3):460–465
22. Saegusa M, Hashimura M, Yoshida T, Okayasu I (2001) β-Catenin mutations and aberrant nuclear expression during endometrial tumorigenesis. Br J Cancer 84(2):209–217
23. Ayhan A, Mao TL, Suryo RY, Zeppernick F, Ogawa H, Wu RC, Wang TL, Shih I (2015) Increased proliferation in atypical hyperplasia/endometrioid intraepithelial neoplasia of the endometrium with concurrent inactivation of ARID1A and PTEN tumour suppressors. J Pathol Clin Res 1(3):186–193
24. Pallares J, Bussaglia E, Martinez-Guitarte JL, Dolcet X, Llobet D, Rue M, Sanchez-Verde L, Palacios J, Prat J, Matias-Guiu X (2005) Immunohistochemical analysis of PTEN in endometrial carcinoma: a tissue microarray study with a comparison of four commercial antibodies in correlation with molecular abnormalities. Mod Pathol 18(5):719–727
25. Fleming GF, Filiaci VL, Marzullo B, Zaino RJ, Davidson SA, Pearl M, Makker V, Burke JN, Zweizig SL, Van Le L, Hanjani P, Downey G, Walker JL, Reyes HD, Leslie KK (2014) Temsirolimus with or without megestrol acetate and tamoxifen for endometrial cancer: a gynecologic oncology group study. Gynecol Oncol 132(3):585–592
26. Visser N, Reijnen C, Massuger L, Nagtegaal ID, Bulten J, Pijnenborg J (2017) Accuracy of endometrial sampling in endometrial carcinoma: a systematic review and meta-analysis. Obstet Gynecol 130(4):803–813
27. Yang S, Thiel KW, Leslie KK (2011) Progesterone: the ultimate endometrial tumor suppressor. Trends Endocrinol Metab 22(4):145–152
28. Smuc T, Rizner TL (2009) Aberrant pre-receptor regulation of estrogen and progesterone action in endometrial cancer. Mol Cell Endocrinol 301(1–2):74–82
29. Xu Y, Tong J, Ai Z, Wang J, Teng Y (2012) Epidermal growth factor receptor signaling pathway involved in progestin-resistance of human endometrial carcinoma: in a mouse model. J Obstet Gynaecol Res 38(12):1358–1366
30. Simpson AN, Feigenberg T, Clarke BA, Gien LT, Ismiil N, Laframboise S, Massey C, Ferguson SE (2014) Fertility sparing treatment of complex atypical hyperplasia and low grade endometrial cancer using oral progestin. Gynecol Oncol 133(2):229–233
31. Ramirez PT, Frumovitz M, Bodurka DC, Sun CC, Levenback C (2004) Hormonal therapy for the management of grade 1 endometrial adenocarcinoma: a literature review. Gynecol Oncol 95(1):133–138
32. Reyes HD, Carlson MJ, Devor EJ, Zhang Y, Thiel KW, Samuelson MI, McDonald M, Yang S, Stephan JM, Savage EC, Dai D, Goodheart MJ, Leslie KK (2016) Downregulation of FOXO1

mRNA levels predicts treatment failure in patients with endometrial pathology conservatively managed with progestin-containing intrauterine devices. Gynecol Oncol 140(1):152–160

33. van Gent MD, Nicolae-Cristea AR, de Kroon CD, Osse EM, Kagie MJ, Trimbos JB, Hazelbag HM, Smit VT, Bosse T (2016) Exploring morphologic and molecular aspects of endometrial cancer under progesterone treatment in the context of fertility preservation. Int J Gynecol Cancer 26(3):483–490