Original Article

DOI: 10.4274/anajog.galenos.2024.09719

Anat J Obstet Gynecol Res 2024;1:25-31

PAX2 and Bcl-2 Expression in Early Stage Endometrioid Adenocarcinoma Compared to Endometrial Hyperplasia and the Relationship with Other Prognostic Factors

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ABSTRACT

Purpose: To determine the tissue expression of Bcl-2 and PAX2 in samples from patients with endometrial hyperplasia and early stage endometrial cancer endometrioid endometrial adenocarcinoma and the relationship with other prognostic factors.

Methods: Patients with early stage endometrial cancer endometrial adenocarcinoma (the cancer group) and with benign endometrial hyperplasia (the hyperplasia group) diagnosed at a single center between 2009 to 2012 and followed up for at least five years were included. Immunohistochemical staining for Bcl-2 and PAX2 was performed. Microcystic, elongated and fragmented pattern (MELF) numbers and ratios were determined by histopathological re-evaluation of slides from samples from the cancer group. Control and patient groups were compared in terms of Bcl-2 and PAX2 scores. Comparison of Bcl-2 and PAX2 scores in relation to different prognostic factors, such as tumor size, myometrial invasion, lymphovascular stromal invasion, myometrial invasion pattern and in tissues neighboring the cancer tissues were performed.

Results: Mean PAX2 expression scores (0.58±1.3 vs 4.3±1.06, $p<0.01$) were significantly lower in cancer group (n=57) compared to the hyperplasia group (n=34). Similarly, mean Bcl-2 expression scores were significantly lower in the cancer group (2.2±1.6 vs 4.06±1, $p<0.01$). MELF pattern was positive in 60% of the cases in cancer group compared to 10.6% in the hyperplasia group ($p<0.01$). Median Bcl-2 scores were higher in MELF positive cases (M=3) compared to MELF negative cases (M=2, $p=0.04$). In addition, median PAX2 scores were higher in MELF positive cases compared to MELF negative cases [2 (range here) vs 0 (range here), $p=0.007$].

Conclusion: Bcl-2 and PAX2 appear to be important in differentiating early stage endometrial cancer endometrioid cancer tissue from hyperplastic endometrial tissue. Their expressions were found to be similar in different prognostic subgroups.

Keywords: Endometrial cancer, Bcl-2, PAX2, gene expression

INTRODUCTION

Adenocarcinoma of the endometrium is the most common malignancy of the female genital system. It is the sixth most common malignancy in women, after breast, colorectal cancers, lung cancer, cervix uteri cancer and thyroid cancer and 4.5% of women are diagnosed with endometrial cancer.[1] Poor prognostic factors for this cancer are advanced surgical stage, advanced age, histological type, advanced tumor grade, presence of myometrial invasion, presence of lymphovascular area invasion, positive peritoneal cytology for cancer cells, and increased tumor size.[2]

Endometrial cancer is detected at stage I in 75% of patients.[3] The survival rate in stage I tumors has been reported as 85-90%. However, patients with early-stage and good prognosis who do not require adjuvant therapies, such as radiotherapy or chemotherapy, have approximately 10-15% recurrence risk during follow-up. These patients respond poorly to adjuvant chemotherapy and some patients are lost due to illness. Unfortunately, the treatment of relapses is challenging. There is a need for easy-to-use, cheap, and rapid prognostic markers to demonstrate poor prognosis in the evaluation of these patients.

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Received: 13.03.2024 **Accepted:** 17.04.2024

Recently, the development of investigation of genomic factors, especially immunohistochemical evaluation of changes in the expression of transcription factors, seem to be promising in determining poor prognosis and for the detection of patients in need of adjuvant therapy.[4] Two immunohistochemical methods have been studied recently which are based on the detection of changes in the expression of the protein products of the *PAX2* and *Bcl-2* genes.[5,6]

The *PAX2* gene is a nuclear transcription factor of the paired box gene family, which is necessary for the development and differentiation of epithelial and mesenchymal components of the urogenital system. Embryonic PAX2 expression is involved in the development of kidney, ureters and renal collecting duct cells, and in women the endometrium, and in men the development of vas deferens and epididymis. Decreased PAX2 expression has been reported to be associated with cervical and endometrial malignant transformation.[7] Interestingly, PAX2 has been found to act as a tumor suppressor during cell proliferation and regeneration in endometrial epithelial cells. Decreased PAX2 expression is associated with endometrial cancer.[8]

Another immunohistochemical marker of the *Bcl-2* gene is one of the main components of the mitochondrial pathway and it plays an anti-apoptotic role in cells.[9] *Bcl-2* may be the most hormone-dependent gene in endometrium and *Bcl-2* gene family members have a significant effect on carcinogenesis. Overexpression of the *Bcl-2* gene results in breast, colon, thyroid, and endometrial cancers. In recent years, there has been evidence that Bcl-2 expression plays an important role in cancer progression and prognosis.[10,11]

The aim of this study was to determine the expression of *PAX2* and *Bcl-2* genes in grade 1 endometrial endometrioid cancer compared to endometrial hyperplasia and the relationship of the levels of expression with poor prognostic factors for endometrial cancer survival.

METHODS

Patients with grade 1 endometrial endometrioid adenocarcinoma attending a single tertiary university center between 1st January 2009 and 31st December 2012 were reviewed for five-year survival rate up to 2018 and constituted the cancer group in this study. Patients diagnosed with benign hyperplasia on histopathological examination of endometrial samples formed the hyperplasia group. The patients' data were collected from the gynecology and pathology files. Specimens of all patients from both groups were re-examined in the light of the current literature, biopsies, and clinical and radiological data. A written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki This study was supported by the local research projects coordination unit as a scientific research project and approved by the Kocaeli University Non-Interventional Research Ethics Committee (dated 19.10.2016 and numbered KÜ GOKAEK 2016/ 287).

The study retrospectively reviewed demographic features of patients, including age, the presence of diabetes, hypertension, and history of accompanying malignancy or relevant family medical history. Pathology reports were also examined to extract surgical stages, tumor size, peritoneal cytology, lymphovascular space invasion, and lymph node metastasis. Patients who received chemotherapy, radiotherapy, or brachytherapy after surgery were identified.

Microcystic, elongated, and fragmented pattern (MELF) ratios and MELF numbers were determined by re-evaluation of the preparations in cancer group. Histopathological findings of surrounding endometrial tissue and invasion patterns in the presence of myometrial invasion were reviewed.

The cancer and hyperplasia groups were compared in terms of Bcl-2 and PAX2 scores. Cross sections with a 4-micron thickness which derived from selected blocs were investigated by using positively charged lamella. After deparaffinizing with xylene, the sections were gradually rehydrated by immersion in changing concentration of ethanol solutions. Antigen retrieval was performed at 100 ˚C for 4 minutes in sodium citrate buffer solution (pH=6). Endogenous peroxidase activity was prevented by immersing the sections in 3% hydrogen peroxide (H_2O_2) solution for 15 minutes. The sections were then crosslinked for 20 hours with human anti-Bcl-2 antibodyat 1/50 dilution (Bcl-2alpha Ab-1 clone, LabVision) and human anti-PAX2 at 1/20 dilution (PAX2, Zeta) antibodies. After incubation, the primary antibody was visualized using streptavidin-biotin complex (UltraVision Detection System Large Volume Anti-Polyvalent, HRP, LabVision) and chromogen (AEC Substrate System, LabVision). Immunohistochemical studies and staining specimens were evaluated by histopathologists. Immunoreactivity was scored semi-quantitatively based on the intensity and distribution of staining for Bcl-2 and PAX2. The staining intensity of the tumor was compared to normal endometrial gland and noted as "increased, decreased, or same" when present. Coloring for the intensity of staining in the gland epithelium were given 0 points for non-staining, 1 point for weak staining, 2 points for moderate staining, and 3 points for strong staining. For the prevalence of staining in the gland epithelium was given 0 for non-staining, 1 point for 1-33% of the gland epithelium, 2 points for 34-66% of the gland epithelium, 3 points for 67-100% of the gland epithelium. The scores given for each case were collected and a final score was determined between 0 and 6 (without 1 point). Also, tumor staining in the surrounding endometrial tissue was categorized as 'increased', 'decreased', or 'unchanged'.

Statistical Analysis

Data were evaluated using SPSS for Windows, version 29 (IBM Inc., Armonk, NY, USA). Nominal variables were evaluated with Pearson's chi-square or Fisher's Exact test. Bcl-2 and PAX2 immunohistochemical staining scores between the control group and cancer group were compared with the Mann-Whitney U test. The between group comparison of Bcl-2 and PAX2 with other prognostic factors was evaluated by Mann-Whitney U and Kruskal-Wallis tests. The relationship between continuous variables with abnormal distribution were evaluated by the Spearman Correlation test and the Pearson correlation test was performed in continuous variables with normal distribution. A $p<0.05$ was considered statistically significant.

RESULTS

There were 57 women in the cancer group and 34 women in the hyperplasia group. Demographic data of the patients are shown in Table 1. Parity, smoking, and diabetes frequency were similar in the cancer and hyperplasia groups. However, the patients in the cancer group were significantly older and more likely to be postmenopausal and hypertensive.

In the cancer group the treatment method consisted of a total abdominal hysterectomy and bilateral salpingo-oophorectomy in all cases. Pelvic lymph node dissection was carried out in 29 (50.8%) patients, while pelvic and para-aortic lymph node dissection was performed in 13 (22.8%) patients. In 28 (49.1%) patients, lymph node dissection was not carried out due to the tumor being limited in the endometrium and the myometrial invasion being less than ½. For patients who underwent lymph node dissection, the number of lymph nodes removed ranged from 0 to 37, with a mean of 15±7.7. In our cohort, 84.2% were stage 1A and there was remission without additional treatment. During five years of follow-up, 2 (3.5%) died due to non-cancerous chronic disease, resulting in 96.4% survival rate and there was one case of recurrence (1.75%).

On review of the pathology results, 35 (61.4%) patients had a tumour size >1 cm and 22 (38.5%) patients has a tumor size <1 cm. Examination of the peritumoral endometrium showed 27 (47.4%) with endometrial atrophy, one (1.75%) with benign hyperplasia, 25 (43.9%) with complex atypical hyperplasia, one (1.75%) with endometrium showing features of progesterone effect and one (1.75%) with secretory endometrium.

The tumor tissue invasion pattern showed an infiltrative pattern in 18 (31.6%), 26 (45.6%) patients with an expansile pattern, one (1.75%) patient with an expansible-infiltrative pattern, and one (1.75%) patient with a destructive pattern. In addition, the MELF pattern was observed in 11 patients. Ten patients had lymphovascular invasion and 60% of them were positive for MELF pattern compared to 10.6% (n=5/47) of the cases without lymphovascular invasion (p<0.01).

The comparison of Bcl-2 and PAX2 scores in cancer vs hyperplasia groups are given in Table 2. Both the PAX2 and Bcl-2 scores were significantly lower in the cancer group compared to the hyperplasia group. Bcl-2 and PAX2 immunohistochemical staining scores with respect to tumor features are presented in Table 3. MELF pattern was positive in 60% of the cases in cancer group compared to 10.6% in the hyperplasia group (p<0.01). Median Bcl-2 scores were higher in MELF positive cases compared to MELF negative cases (p=0.04). Median PAX2 scores were higher in MELF positive cases (M=2) compared to MELF negative cases (p=0.007). Interestingly, Bcl-2 and PAX2 scores were similar in lymphovascular stromal invasion positive and negative cases, in cases with different myometrial invasion depth and different tumor size. Bcl-2 scores were similar in different myometrial invasion patterns and hyperplastic or atrophic tissue surrounding the cancer tissue. PAX2 scores were significantly higher in tumors exhibiting an infiltrative-type myometrial invasion pattern compared to expansive pattern (p=0.03). In the cancer group, hyperplastic tissue surrounding the cancer tissue had a higher PAX2 score compared to surrounding atrophic tissue (p=0.03). Although positive or negative LVSI and MELF pattern showed no significant difference in Bcl-2 staining for tumor and surrounding endometrium, Bcl-2 staining was significantly increased in the presence of more than half depth myometrial invasion (p=0.04) (Table 4). PAX2 staining was found to be similar both in tumor tissue and the surrounding endometrium in terms of LVSI and MELF pattern regardless of being positive or negative, myometrial invasion and invasion pattern (Table 5).

Table 1. Demographic data of patients with endometrial cancer and endometrial hyperplasis

Parameters	Cancer group (n=57)	Hyperplasia group (n=34)	p-value
Mean ± SD age, (years)	58.7±8.5	45.5±7.25	<0.01
Mean ± SD parity	3.3±1.87	3±1.39	0.709
Smoking, n (%)	5 (8.8)	2 (5.9)	0.617
Diabetes, n (%)	21 (36.8)	11 (32.4)	0.664
Hypertension, n (%)	35 (61.4)	13 (8.6)	0.03
Menopause status, n (%)			
Premenopausal	1 (1.8)	16 (47.1)	<0.01
Perimenopausal	15 (26.3)	11 (32.4)	
Post-menopausal	36 (63.2)	7 (20.6)	
SD: Standard deviation			

DISCUSSION

The results of the present study suggest that Bcl-2 and PAX2 expression scores can be used to differentiate early stage, well differentiated endometrioid endometrial cancers from

Table 2. Comparison of Bcl-2 and PAX2 scores in the endometrial cancer vs endometrial hyperplasia groups

Variable	Cancer group (n=57)	Hyperplasia group (n=34)	p-value
PAX2 score			
Median (min-max)	0.5 (0-5)	4 (2-6)	<0.01
Mean ± SD	0.58±1.3	4.3±1.065	
Bcl-2 score			
Median (min-max)	2 (0-6)	4 (0-6)	<0.01
Mean ± SD	2.2±1.6	4.06±1	
SD: Standard deviation, min-max: Minimum-maximum			

Table 3. Bcl-2 and PAX2 immunohistochemical staining scores with respect to tumor features

Tumor features	Bcl-2 score Median (min-max)	p-value	PAX2 score Median (min-max)	p-value
Tumor size				
Less than 1 cm (n=22)	2 (0-6)	0.59	0.5 (0-4)	0.98
More than 1 cm (n=35)	2 (0-5)		0	
Myometrial invasion				
No invasion (n=10)	2 (0-6)	0.83	0.6 (0-4)	0.36
<half depth (n=38)	2 (0-6)		0 (0-4)	
>half depth (n=9)	3 (0-4)		0 (0-5)	
LVI				
Positive (n=10)	2 (0-4)	0.73	0 (0-5)	0.26
Negative (n=47)	2 (0-6)		0 (0-4)	
MELF				
Positive (n=11)	3 (2-4)	0.04	2 (0-5)	0.007
Negative (n=46)	2 (0-6)		0 (0-4)	
Myometrial invasion pattern				
Expansive (n=28)	2 (0-6)	0.48	0 (0-4)	0.03
Infiltrative (n=19)	3 (0-4)		1 (0-5)	
Surrounding endometrium				
Hyperplastic (n=28)	3 (0-5)	0.35	1 (0-4)	0.03
Atrophic (n=29)	2 (0-6)		0 (0-5)	

LVI: Lymphovascular invasion, MELF: Microcystic, elongated and fragmented pattern, min-max: Minimum-maximum

Table 4. Bcl-2 staining comparison between the tumor and the surrounding endometrium

	Bcl-2 comparison			p-value
	Increased n (%)	Decreased n (%)	Sam n (%)	
LVI				
Negative	2 (4.3)	43 (91.5)	2 (4.3)	0.384
Positive	1 (12.5)	6 (75)	1 (12.5)	
MELF				
Positive	1 (9.1)	9 (81.9)	1 (9.1)	0.688
Negative	2 (4.5)	40 (90.9)	2 (4.5)	
Myometrial invasion				
Negative	1 (10)	9 (90)	0	0.04
<half depth	1 (2.8)	34 (94.4)	1 (2.8)	
>half depth	1 (11.1)	6 (66.7)	2 (22.2)	
Invasion pattern				
Infiltrative	1 (5.9)	13 (76.5)	3 (17.6)	0.07
Expansive	1 (3.8)	25 (96.2)	0	
Surrounding endometrium				
Hyperplastic	1 (4.1)	23 (95.9)	0 (0)	0.187
Atrophic	2 (7.7)	21 (80.8)	3 (11.5)	

LVI: Lymphovascular invasion, MELF: Microcystic, elongated and fragmented pattern

Table 5. PAX2 staining comparison of tumor tissue to surrounding endometrium

	PAX2 comparison		p-value
	Increased n (%)	Decreased n (%)	
LVI			
Negative	0 (0)	47 (100)	0.14
Positive	1 (12.5)	7 (87.5)	
MELF			
Positive	1 (9.1)	10 (90.9)	0.2
Negative	0 (0)	44 (100)	
Myometrial invasion			
Negative	0 (0)	10 (100)	0.08
<half depth	0 (0)	36 (100)	
>half depth	1 (11.1)	8 (88.9)	
Invasion pattern			
Infiltrative	1 (5.9)	16 (94.1)	0.211
Expansive	0 (0)	26 (100)	
Surrounding endometrium			
Hyperplastic	0 (100)	24 (100)	0.332
Atrophic	1 (3.8)	25 (96.2)	

LVI: Lymphovascular invasion, MELF: Microcystic, elongated and fragmented pattern

endometrial hyperplasia. Endometrial cancer is the most common malignancy of the female genital system. Reduction of cervical cancer rates by population screening programs has increased the clinical importance of endometrial cancer. In the United States, 65,950 new cases of endometrial cancer are estimated to occur in 2022, with 12,550 resulting deaths.[12] In 2016, a multicenter study from Turkey reported endometrial cancer as the fourth most common cancer among women.[13]

Stage is the most important prognostic factor in endometrial cancer. In a cohort study published by Jeppesen et al.,[14] 5-year life expectancy in early-stage endometrial cancers was 87.5% for stage 1A, 77.5% for stage 1B, and 69% for stage 2. In the present study, 84.2% of the patients were found to be stage 1A and the disease resulted in remission without additional treatment. During five years of follow-up, there was a 96.4% survival rate and no patient died as a result of their endometrial cancer.

Many genetic mutations, resulting in PTEN inactivation or changed E-cadherin expression, or mutations in *p53* or *K-ras* have been studied in endometrial cancer.[15-19] There are conflicting results regarding Bcl-2 expression in endometrial tissues, but the general view is that Bcl-2 expression is decreased in endometrial cancer.[19,20] This view was supported by our findings with Bcl-2 expression significantly decreased in endometrial cancer patients compared to patients with endometrial hyperplasia.

In 2015, Stewart and Crook reported that loss of PAX2 expression in endometrial cancer was associated with poor prognosis.[21] In the same year, Joiner et al.[22] examined PAX2 expression in relation to World Health Organization (WHO) and endometrial intraepithelial neoplasia (EIN) classification of endometrial neoplasms and found no correlation between WHO 1994 classification and PAX2 expression. However, a decrease in PAX2 expression was associated with EIN status in patients. These authors suggested that decreased PAX2 expression can be used as a marker for progression to malignancy.[22] Kahraman et al.[23] examined the expression of PAX2 in hyperplastic and cancerous endometrial tissues in 2012 and reported that PAX2 expression was increased in cancerous tissue, which is in contrast to our findings. Quick et al.[24] studied PAX2 expression in patients with EIN and found that the reduction of PAX2 expression was valuable in the diagnosis of EIN, premalignant lesions that are precursors of endometrial cancer. In the present study, PAX2 expression was significantly reduced in cancerous tissues compared to healthy control tissues. However, when compared with other prognostic factors, there was no significant difference in those, with the exception of MELF pattern. Increased PAX2 expression was found in MELF positive cases. Increased Bcl-2 expression scores were associated with MELF pattern, which is one of the criteria that has been considered pathologically in recent years. MELF pattern was also found to be more frequent in cases with lymphovascular stromal invasion. In the present study, Bcl-2 and PAX2 scores were higher in MELF-positive patients.

Lymphovascular invasion is associated with recurrence and mortality in early stage grade 1 tumors, regardless of histologic types of endometrial cancer. In a study by Hanson et al.,[25] the rate of lymphovascular invasion was reported as 2% in grade 1 tumors, 5% in superficial myometrial invasion, 42% in grade

3 tumors and 70% in deep myometrial invasion. In the present study, lymphovascular invasion was not present in any patients without there also being myometrial invasion. Furthermore, lymphovascular invasion was found in 10.5% with less than half the depth of myometrial invasion but present in 66.7% of patients with myometrial invasion deeper than half the depth of the myometrium.

Tumor size is known to be one of the factors affecting prognosis in endometrial cancer. Senol et al.[26] reported that tumors smaller than 2 cm had a lower risk of lymph node spread (4%), whereas this rate increased to 15% in tumors larger than 2 cm. If the tumor covers the entire uterine cavity, the risk of lymph node metastasis was as high as 35%. Similarly, 5-year survival rate was 98% for tumors smaller than 2 cm, 84% for tumors larger than 2 cm, and 64% for tumors filling the entire uterine cavity.[26] In the present study, there were 22 patients with tumor size smaller than 1 cm, and 35 patients with tumor size larger than 1 cm. When Bcl-2 scores and PAX2 expression were compared with respect to tumor size, there was no significant difference.

Myometrial invasion is a prognostic factor that changes stage in endometrial cancer and also decreases life expectancy with lymph node metastasis.[27] Kaku et al.[28] in a study of non-invasive or superficial invasive tumors reported that the 90% 5-year survival rate was reduced to 60% when there was deep myometrial invasion. In the light of this, we compared myometrial invasion and Bcl-2 scores. However, we found no difference in Bcl-2 and PAX2 expression and myometrial invasion status.

Our findings should be considered in the light of the following, which may be considered limitations. There was a high rate of 5-year survival in patients with grade 1 endometrioid adenocarcinoma, there is good prognosis with this diagnosis, there were no patients who died primarily from endometrial cancer, and only one patient had recurrence of the disease.

CONCLUSION

Recently, developing genomic factors, especially immunohistochemical evaluation of changes in expression of transcription factors has been suggested as being useful when considering prognosis and for identifying patients who require adjuvant therapy. Recently, two immunohistochemical methods, based on the detection of changes in the protein expression of the *PAX2* and *Bcl-2* genes have been described. While the results of the present study suggest that Bcl-2 and PAX2 protein expression scores appear to be useful for separating cancer tissue from benign hyperplastic tissues, more studies should be performed before these biomarkers can be incorporated in routine diagnosis. However, these developments look promising for the future clinical management of endometrial cancer and may be used to determine the requirement for both surgical planning and post-surgical adjuvant methods by evaluating endometrial specimens using immunohistochemical techniques.

Ethics

Ethics Committee Approval: Approval for this study was received from Kocaeli University Non-Interventional Research Ethics Committee (dated 19.10.2016 and numbered KÜ GOKAEK 2016/ 287).

Informed Consent: A written informed consent was obtained from each patient.

Authorship Contributions

Surgical and Medical Practices: M.K., L.A., A.Ç, Concept: M.K., H.D., B.A., A.Ç, Design: M.K., B.A., A.Ç, Data Collection or Processing: M.K., M.Ç.K., L.A., H.D., Analysis or Interpretation: M.K., M.Ç.K., H.D., A.Ç, Literature Search: M.K., M.Ç.K., L.A., B.A., Writing: M.K., L.A., H.D., B.A., A.Ç.

Conflicts of Interest: The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249.
2. Zusterzeel PL, Bekkers RL, Hendriks JC, Neesham DN, Rome RM, Quinn MA. Prognostic factors for recurrence in patients with FIGO stage I and II, intermediate or high risk endometrial cancer. Acta Obstet Gynecol Scand. 2008;87(2):240-246.
3. Guntupalli SR, Zighelboim I, Kizer NT, et al. Lymphovascular space invasion is an independent risk factor for nodal disease and poor outcomes in endometrioid endometrial cancer. Gynecol Oncol. 2012 Jan;124(1):31-35.
4. Niu S, Molberg K, Castrillon DH, Lucas E, Chen H. Biomarkers in the Diagnosis of Endometrial Precancers. Molecular Characteristics, Candidate Immunohistochemical Markers, and Promising Results of Three-Marker Panel: Current Status and Future Directions. Cancers. 2024;16(6):1159.
5. Travaglino A, Raffone A, Saccone G, et al. Immunohistochemical predictive markers of response to conservative treatment of endometrial hyperplasia and early endometrial cancer: A systematic review. Acta Obstet Gynecol Scand. 2019;98(9):1086-1099.
6. Patrício P, Ramalho-Carvalho J, Costa-Pinheiro P, et al. Deregulation of PAX2 expression in renal cell tumours: mechanisms and potential use in differential diagnosis. J Cell Mol Med. 2013;17(8):1048-1058.
7. Zhang HS1, Yan B, Li XB, et al. PAX2 protein induces expression of cyclin D1 through activating AP-1 protein and promotes proliferation of colon cancer cells. J Biol Chem. 2012;287(53):44164-44172.
8. Zhang LP, Shi XY, Zhao CY, Liu YZ, Cheng P. RNA interference of pax2 inhibits growth of transplanted human endometrial cancer cells in nude mice. Chin J Cancer. 2011;30(6):400-406.
9. Devis-Jauregui L, Eritja N, Davis ML, Matias-Guiu X, Llobet-Navàs D. Autophagy in the physiological endometrium and cancer. Autophagy. 2021;17(5):1077-1095.
10. Linjawi A, Kontogiannea M, Halwani F, Edwardes M, Meterissian S. Prognostic significance of p53, bcl-2, and Bax expression in early breast cancer. J Am Coll Surg. 2004;198(1):83-90.
11. Zhang R, He Y, Zhang X, et al. Estrogen receptor-regulated microRNAs contribute to the BCL2/BAX imbalance in endometrial

adenocarcinoma and precancerous lesions. Cancer Lett. 2012;314(2):155-165.

12. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7-33.
13. Anton-Culver H, Chang J, Bray F, et al. Cancer burden in four countries of the Middle East Cancer Consortium (Cyprus; Jordan; Israel; Izmir (Turkey)) with comparison to the United States surveillance; epidemiology and end results program. Cancer Epidemiol. 2016;44:195-202.
14. Jeppesen MM, Jensen PT, Gilså Hansen D, Iachina M, Mogensen O. The nature of early-stage endometrial cancer recurrence-A national cohort study. Eur J Cancer. 2016;69:51-60.
15. Salvesen HB, MacDonald N, Ryan A, et al. PTEN methylation is associated with advanced stage and microsatellite instability in endometrial carcinoma. Int J Cancer. 2001;91(1):22-26.
16. Saito T, Nishimura M, Yamasaki H, Kudo R. Hypermethylation in promoter region of E-cadherin gene is associated with tumor dedifferention and myometrial invasion in endometrial carcinoma. Cancer. 2003;97(4):1002-1009.
17. Tashiro H, Isacson C, Levine R, Kurman RJ, Cho KR, Hedrick L. p53 gene mutations are common in uterine serous carcinoma and occur early in their pathogenesis. Am J Pathol. 1997;150(1):177-185.
18. Matias-Guiu X, Catasus L, Bussaglia E, et al. Molecular pathology of endometrial hyperplasia and carcinoma. Hum Pathol. 2001;32(6):569-577.
19. Liu FS. Molecular carcinogenesis of endometrial cancer. Taiwan J Obstet Gynecol. 2007;46(1):26-32.
20. Semczuk A, Schneider-Stock R, Berbec H, Marzec B, Jakowicki JA, Roessner A. K-ras exon 2 point mutations in human endometrial cancer. Cancer Lett. 2001;164(2):207-212.
21. Stewart CJ, Crook ML. PAX2 and cyclin D1 expression in the distinction between cervical microglandular hyperplasia and endometrial microglandular-like carcinoma: a comparison with p16, vimentin, and Ki67. Int J Gynecol Pathol. 2015;34(1):90-100.
22. Joiner AK, Quick CM, Jeffus SK. Pax2 expression in simultaneously diagnosed WHO and EIN classification systems. Int J Gynecol Pathol. 2015;34(1):40-46.
23. Kahraman K, Kiremitci S, Taskin S, Kankaya D, Sertcelik A, Ortac F. Expression pattern of PAX2 in hyperplastic and malignant endometrium. Arch Gynecol Obstet. 2012;286(1):173-178.
24. Quick CM, Laury AR, Monte NM, Mutter GL. Utility of PAX2 as a marker for diagnosis of endometrial intraepithelial neoplasia. Am J Clin Pathol. 2012;138(5):678-684.
25. Hanson MB, van Nagell JR Jr, Powell DE, et al. The prognostic significance of lymph-vascular space invasion in stage I endometrial cancer. Cancer. 1985;55(8):1753-1757.
26. Senol T, Polat M, Ozkaya E, Karateke A. Tumor Diameter for Prediction of Recurrence, Disease Free and Overall Survival in Endometrial Cancer Cases. Asian Pac J Cancer Prev. 2015;16(17):7463-7466.
27. Nwachukwu C, Baskovic M, Von Eyben R, et al. Recurrence risk factors in stage IA grade 1 endometrial cancer. J Gynecol Oncol. 2021;32(2):e22.
28. Kaku T, Tsuruchi N, Tsukamoto N, Hirakawa T, Kamura T, Nakano H. Reassessment of myometrial invasion in endometrial carcinoma. Obstet Gynecol. 1994;84(6):979-982.