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ORIGINAL ARTICLE

BCL-2 and PAX2 Expressions in EIN which Had Been Previously Diagnosed as Non-Atypical Hyperplasia

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Abstract

The relationship between PAX2 and another anti-apoptotic gene, BCL-2, has been shown in a limited number of studies. The aims of this study are to investigate the value of PAX2 and BCL-2 expressions in lesions which have been defined as nonatypical hyperplasia in terms of detecting EIN and to evaluate the relations of these proteins in EIN. For this purpose, 108 cases of non-atypical endometrial hyperplasia diagnosed from 2006 to 2011 were re-evaluated. Immunohistochemical studies with PAX2 and BCL-2 were performed in 20 cases with EIN and 34 cases with benign hyperplasia. The mean BCL-2 immunohistochemistry scores of benign hyperplasia and EIN cases were 4.06 ± 1.04 and 4.63 ± 2.03, respectively. The mean BCL-2 score of EIN cases was significantly higher than benign hyperplasia ($p = 0.021$). The mean PAX2 scores of benign hyperplasia and EIN cases were 4.32 ± 1.07 and 2.19 ± 2.34, respectively. The mean PAX2 scores of EIN cases were significantly lower than benign hyperplasia ($p = 0.001$). BCL-2 expression was increased compared to normal endometrium in 66.7% of EIN cases, and PAX2 expression was decreased in 73.3%. Consistent with this, in 60% of cases, BCL-2 expression was increased compared to normal endometrium, while PAX2 expression was decreased. BCL-2 and PAX2 protein expression changes occur in early phases of endometrial tumorigenesis. These changes are often seen as a simultaneous increase in BCL-2 expression and decrease in PAX2 expression.

Keywords Endometrial intraepithelial neoplasia · PAX2 · BCL-2 · Immunohistochemistry · Uterus · Endometrial hyperplasia

Introduction

The most widely used classification for precursor lesions of endometrioid carcinoma (EC) is the system proposed by Kurman et al. in 1985, and published by the World Health Organization (WHO) in 1984 [1]. In this classification, a distinction made based on structural and cytological features of these lesions, but not the molecular background. Unlike this classification, which can be used very easily in everyday use, the investigation of clonality is an expensive method that can be performed in certain centers.

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As molecular methods have developed, the pathogenesis of precursor lesions of EC has been elucidated. Precursor lesions have been shown to be monoclonal proliferations, such as cancers [2–4]. Gene mutations -such as *KRAS* and *PTEN*- which are thought to be responsible for monoclonal proliferation have been identified at early stages of endometrial cancer development [5].

BCL-2 is a member of a gene family with the same name. It is a gene with well-known anti-apoptotic effects [6]. There are many studies investigating the expression of this gene's product -BCL-2 protein- in precursor lesions of EC. Although there are different opinions on these studies, decreased BCL-2 expression in atypical hyperplasia and EC is widely accepted [7–9].

PAX2 is a member of the *PAX* gene family and is associated with renal development during embryogenesis [10, 11]. The protein -PAX2- produced by this gene is a proto-oncogenic anti-apoptotic protein. There are a number of studies showing decreased expression of PAX2 in endometrial cancers and its precursor lesions [12, 13].

Both *BCL-2* and *PAX2* are anti-apoptotic and proto-oncogenic genes, but there are a few studies that show how they affect each other's function. Winyard et al. showed a high level of expression of PAX2 and BCL-2 in the first phase of

nephron development [14]. Zhang et al. showed increased necrosis in endometrial cancer cell culture in which *PAX2* is inhibited by siRNA; BCL-2 expression is decreased in these cells [15].

Mutter published five criteria that are easily applied to the diagnosis of precursor lesions of EC and are compatible with clonality findings [16]. The lesions meeting these criteria are defined as endometrial intraepithelial neoplasia (EIN). The WHO accepted this terminology in 2014 [17].

A number of studies which contain the comparison of WHO 1994 and WHO 2014 classifications have been reported [4, 18, 19]. In these studies, EIN has been detected in 47.2–86.5% of cases with atypical hyperplasia and 13.5–20.9% of cases with non-atypical hyperplasia. However, given the absence of classical nuclear atypical features, detection of EIN could be challenging in lesions which have been formerly defined as 'non-atypical hyperplasia', because of wide use and familiarity of previous classification. The aims of this study are to investigate the value of PAX2 and BCL-2 expressions in lesions which have been defined as non-atypical hyperplasia in terms of detecting EIN and to evaluate the relations of these proteins in EIN.

Materials and Methods

Patients

We reviewed 108 cases initially diagnosed with non-atypical endometrial hyperplasia on endometrial biopsy or curettage material between 2006 and 2011 from the Kocaeli University School of Medicine. Endometrial biopsy and curettage specimens of these patients were reviewed and reclassified according to WHO 2014 endometrial hyperplasia classification [17]. EIN criteria published by Mutter were used for describing EIN [16]. All cases were reviewed by one general pathologist (LT) and one gynecological pathologist (BM). In initial evaluation, they were blinded to each other's opinions. Cases with discordant diagnosis were discussed together in second session and final diagnosis for these cases were made.

Immunohistochemistry

For the immunohistochemical study, 20 cases with EIN and 34 random patients without EIN were included. Immunohistochemical staining used anti-BCL-2 (BCL-2alpha Ab-1, clone: 100/D5, 1/50, LabVision, Fremont, CA, USA) and anti-PAX2 (PAX2, clone: EP235, 1/20, Zeta, Arcadia, CA, USA). The immunohistochemical stainings were performed on sections from formalin fixed-paraffin embedded tissues. Immunohistochemical antibody clone names, sources, dilutions and antigen retrieval details were listed in Table 1.

Table 1 Details of immunohistochemical antibodies

Antibody	Manifacturer	Clone	Dilution	Antigen Retrieval
BCL-2	LabVision	100/D5	1/50	EDTA
PAX2	Zeta	EP235	1/20	EDTA

Tonsil and kidney tissues were used as positive controls for BCL-2 and PAX2, respectively. Cytoplasmic reaction for BCL-2 and nuclear reaction for PAX2 were considered as positive.

For the EIN cases, only lesion-specific epithelium was scored. If present, the signal intensity was compared with normal endometrial glands (increased, decreased, not changed). Since there is no consensus on evaluating immunohistochemical expression of PAX2 and BCL-2 in endometrium, we used a modified scoring system based on the method suggested by Allred et al. while evaluating immunohistochemical expression of both of the markers [20]. For this purpose, an intensity score (0; negative, 1; weak, 2; moderate, 3; strong), a proportional score (0; 0, 1; 1–33, 2; 34–66, 3; 67–100%) and a total score (between 0 and 6) were assigned for each slide.

Statistical Analysis

Statistical evaluation used IBM SPSS 22.0 (SPSS Inc., Chicago, IL, USA) package program. The normality test was assessed using the Kolmogorov-Smirnov Test. Mann Whitney U test and Kruskal Wallis one-way analysis of variance were used for numerical variables with no normal distribution. $p < 0.05$ was considered statistically significant.

Results

EIN is detected in 20 cases out of 108 (22.7%). Mean ages of 88 benign hyperplasia and 20 EIN cases were 45.4 (22–72) and 47.6 (36–57), respectively. The initial diagnoses of the 20 cases with EIN were simple hyperplasia in 9, complex hyperplasia in 11 (Table 2).

Immunoreactivity scores could not be assigned for one case for PAX2, one case for BCL-2, and three cases for both stains, since the small EIN areas were lost in IHC stained slides.

Table 2 Distribution of the diagnoses according to WHO 1994 and 2014 classifications

	Benign hyperplasia	EIN	Total
Simple hyperplasia	86	9	95 (88%)
Complex hyperplasia	2	11	13 (12%)
Total	88 (81.5%)	20 (18.5%)	108

Immunohistochemistry

The mean BCL-2 scores of benign hyperplasia and EIN were 4.06 ± 1.04 and 4.63 ± 2.03, respectively (Fig. 1). The mean BCL-2 score of EIN cases was significantly higher than that of benign hyperplasia ($p = 0.021$).

The mean PAX2 scores of benign hyperplasia and EIN cases were 4.32 ± 1.06 and 2.19 ± 2.34, respectively (Fig. 2). The mean PAX2 scores of EIN cases were significantly lower than that of benign hyperplasia ($p = 0.001$).

Of the 15 EIN cases where both IHC stains could be evaluated, 10 (66.7%) had increased BCL-2 expression relative to normal endometrium while 11 (73.3%) had decreased PAX2 expression (Table 3). Consistent with this, 9 (60%) cases had both increased BCL-2 expression and decreased PAX2 expression relative to normal endometrium (Fig. 3) (Table 4).

Patient Outcome

Re-biopsy or hysterectomy had been performed in 10 cases with EIN out of 20 and 40 cases with benign hyperplasia out of 88. Only 1 case with EIN had subsequent EC diagnosis after the initial biopsy. The initial diagnosis for this case was complex hyperplasia and the EIN area had no expression for both PAX2 and BCL-2 proteins. Average time between the initial biopsy and re-biopsy or hysterectomy was 6.4 months (1–39 months) for EIN cases and 13.9 months (1–96 months) for benign hyperplasia.

Discussion

Since 2014, EIN has been described as a precursor lesion of EC. As a number of relevant studies have shown, this terminology expresses that these lesions are monoclonal and have a molecular background [2, 5].

The BCL-2 protein has well-known anti-apoptotic effects. p53 controls the anti-apoptotic effect of BCL-2 in several ways. First, p53 may directly bind to the promoter region of the *BCL-2* gene and inhibit transcription. In addition, it may inhibit BCL-2 production indirectly by activating the transcription of its target genes such as *PUMA* and *NOXA* which inhibit BCL-2 production. BCL-2 activity is also regulated by other cell cycle regulatory genes such as *myc* [21, 22].

There are many studies of BCL-2 expression in precursor lesions of EC but with contrasting results. However, the consensus is that BCL-2 expression is decreased in atypical hyperplasia and EC [7–9]. In addition, Bozkurt et al. showed that there is no difference in BCL-2 expression between EIN and benign endometrium [23]. Amalinei et al. reported that BCL-2 expression was stronger in endometrial hyperplasia and EC than non-neoplastic endometrium [24]. In our study, there was a significant difference between BCL-2 scores of the EIN and benign hyperplasia cases ($p = 0.021$).

PAX2 is a member of the *Pax* gene family and produces a protein with the same name that has anti-apoptotic and proto-oncogenic effects. The anti-apoptotic effects of PAX2 protein are mediated by binding to the regulatory region at the 5′ end of *P53* gene and inhibiting its protein production at the transcriptional level [10, 11]. The PAX2 expression is activated

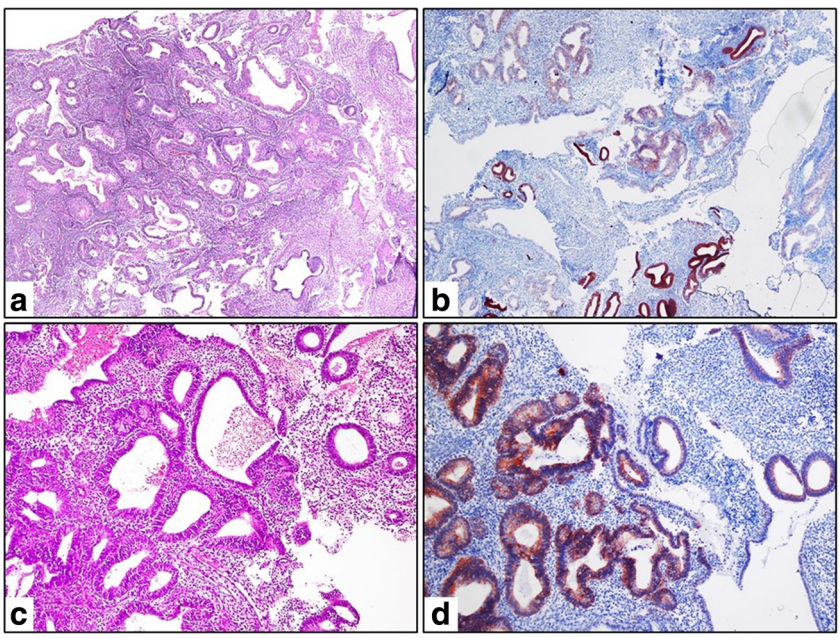

Fig. 1 BCL-2 Expression in EIN. a, b: Decreased BCL-2 expression in EIN compared to normal endometrium (a: H-E ×40, b: IHC ×40); **c, d:** Increased BCL-2 expression in EIN compared to normal endometrium (a: H-E ×100, b: IHC ×100)

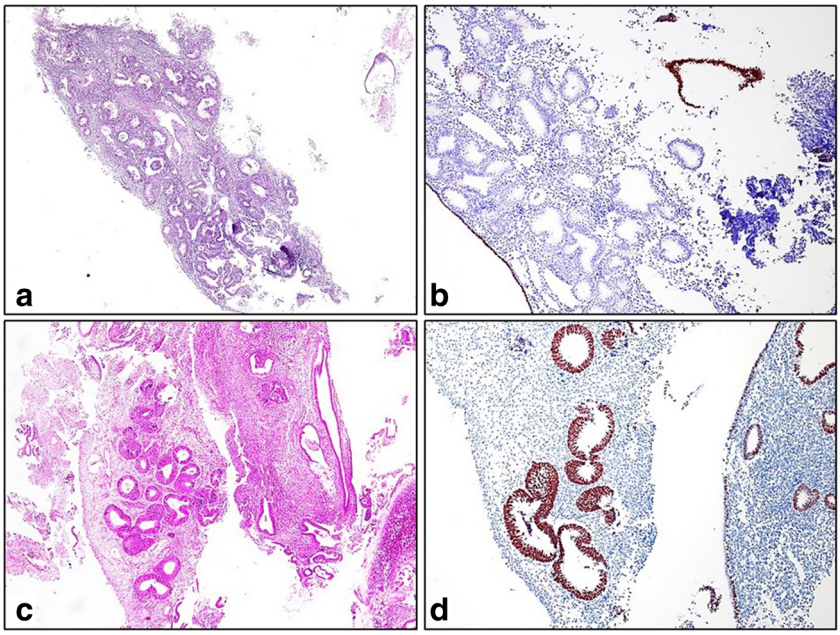

Fig. 2 PAX2 Expression in EIN. **a, b:** Decreased PAX2 expression in EIN compared to normal endometrium (a: H-E ×40, b: IHC ×100); **c, d:** Increased PAX2 expression in EIN compared to normal endometrium (c: H-E ×40, d: IHC ×100)

indirectly by the ERα pathway [25]. EC cell culture studies have shown that PAX2 expression correlates with tumor growth and ERα expression [15, 25]. However, studies on human tissues have shown that PAX2 levels decrease in EC cases. On the other hand, the expression of other proteins activated by ERα pathway increased. Strissel et al. stated that this condition might due to the absence of ERE on the *PAX2* gene [26].

There are a number of studies investigating PAX2 expression in precursor lesions of EC. The common suggestion is that PAX2 expression decreases in EC and its precursors [12, 13]. However, there are a few studies advocating an opposing viewpoint [27]. Joiner et al. reported that PAX2 expression generally decreased in EIN compared to normal endometrium, on the other hand in a small number of cases PAX2 expression elevated [28]. Similar to the literature, we found that 73.3% of EIN cases had decreased PAX2 expression compared to normal endometrium. In addition, we observed that PAX2 expression in three cases (20%) was increased relative to normal endometrium. However, the mean PAX2 scores in cases with EIN was significantly lower than those of benign hyperplasia ($p = 0.001$).

Table 3 Distribution of Bcl-2 and Pax2 scores of EIN cases which are available for evaluation for both stains

Pax2 / Bcl-2	0	2	3	4	5	6	Total
0	2	0	0	0	0	0	2
2	0	0	0	0	0	0	0
3	0	0	0	0	0	1	1
4	0	0	0	0	0	1	1
5	1	1	1	0	0	0	3
6	3	1	2	1	0	1	8
Total	6	2	3	1	0	3	15

Winyard et al. reported high expression of PAX2 and BCL-2 in the early phase of nephron formation in renal development. However, in the branches of ureter buds, there was no BCL-2 expression; proliferation correlated with PAX2 expression. This might be related to the production of other anti-apoptotic molecules (such as Bcl_{XL}) or to low levels of apoptosis-triggering molecules (such as Bax) [14]. Given the data obtained from these studies and the relation of PAX2 and BCL-2 proteins with p53, one may suggest that the production of these two proteins will be in similar directions even in variable situations. However, the mechanisms that regulate the production of both proteins and their effects on the cell cycle are complex and not fully understood.

In our study, the BCL-2 and PAX2 expressions were in inverse directions relative to normal endometrium in 60% of the EIN cases. Although PAX2 loss is a well-known alteration in EIN, elevated expression of BCL-2 is still not clarified. Our findings indicate that these two proteins may be affected diversely in the same gland.

Although expression loss is seen in the majority of EIN cases, there were 3 cases (19%) that had increased PAX2 expression relative to normal endometrium. There are a few publications showing that PAX2 expression may be increased in a small number of cases despite the common conclusion that PAX2 expression is lost in EIN cases [13, 28]. Similarly, 4 cases (25%) had decreased BCL-2 expression relative to normal endometrium although the most of EIN cases had increased BCL-2 expression. These findings suggest that there could be multiple pathways that affect both of these proteins' expressions in endometrial carcinogenesis. These pathways are still waiting to be elucidated.

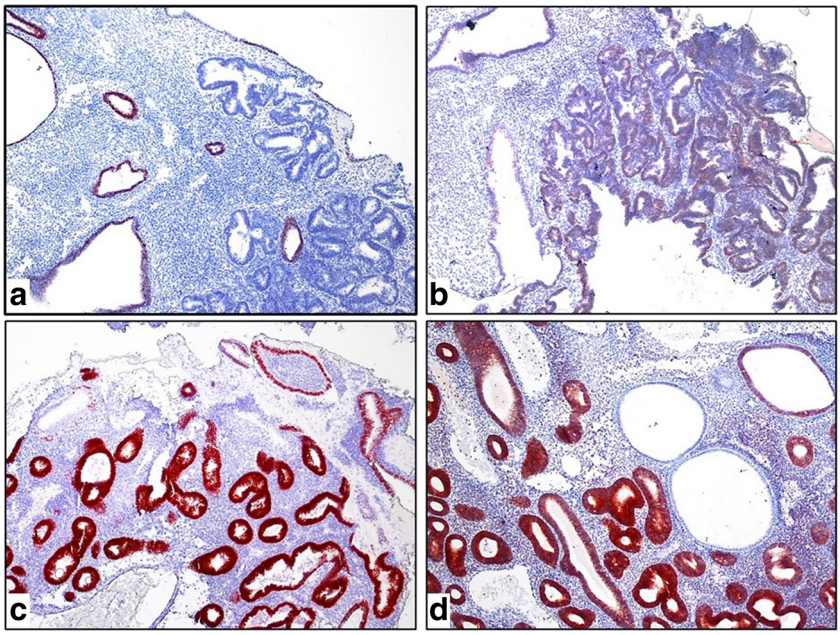

Fig. 3 Relation of BCL-2 and PAX2 Expressions in EIN. a, b: Decreased PAX2 and increased BCL-2 expression in the same lesion compared to normal endometrium (IHC, ×100); **c, d:** Increased PAX2 and increased BCL-2 expression in the same lesion compared to normal endometrium (IHC, ×100)

The major limitation of our study was that only immunohistochemical study was used for protein detection. These results should be confirmed by Western-blot analysis and RT-PCR. As a matter of course, our series had limited numbers of EIN cases because we focused on cases that previously diagnosed as non-atypical hyperplasia.

Quick et al. stated that loss of PAX2 expression can be used as an adjuvant finding for the EIN diagnosis in cases where comparison with normal endometrium is not possible [13]. In support, we observed that PAX2 expression was completely lost in 43.8% of EIN cases in our study. There is no study focusing on the diagnostic value of BCL-2 expression alterations in EIN. Although we detected BCL-2 expression alterations in 87.5% of EIN cases in our study, we conclude that these alterations could not be disclosed objectively without evaluating the accompanying normal endometrium.

Here, we showed that EIN had significantly lower PAX2 and higher BCL-2 expressions than benign hyperplasia in the cases previously diagnosed as non-atypical hyperplasia. We suggest that these joint alterations will be helpful in terms of detecting EIN, especially in a challenging histomorphology which there is uncertain cytological demarcation with volume percentage stroma that is verging on 55%. Besides, we suggest that our findings may also generate another viewpoint in understanding endometrial carcinogenesis, considering the inverse relation of BCL-2 and PAX2 expressions in EIN.

Table 4 BCL-2 and PAX2 alterations in EIN cases which are available for comparison with normal endometrium and are available for evaluation for both stains

Pax2 Bcl-2	Decreased	Not changed	Increased	Total
Decreased	2	0	2	4
Not changed	0	1	0	1
Increased	9	0	1	10
Total	11	1	3	15

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Compliance with Ethical Standards

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

References

1. Kurman RJ, Kaminski PF, Norris HJ (1985) The behavior of endometrial hyperplasia. A long-term study of "untreated" hyperplasia in 170 patients. Cancer 56:403–412
2. Mutter GL, Baak JP, Crum CP et al (2000) Endometrial precancer diagnosis by histopathology, clonal analysis, and computerized morphometry. J Pathol 190:462–469. https://doi.org/10.1002/(SICI)1096-9896(200003)190:4<462::AID-PATH590>3.0.CO;2-D
3. Mutter GL, Chaponot ML, Fletcher JA (1995) A polymerase chain reaction assay for non-random X chromosome inactivation identifies monoclonal endometrial cancers and precancers. Am J Pathol 146:501–508
4. Baak JP, Mutter GL, Robboy SJ et al (2005) The molecular genetics and morphometry-based endometrial intraepithelial neoplasia classification system predicts disease progression in endometrial hyperplasia more accurately than the 1994 World Health Organization classification system. Cancer 103:2304–2312. https://doi.org/10.1002/cncr.21058

5. Hecht JL, Mutter GL (2006) Molecular and pathologic aspects of endometrial carcinogenesis. J Clin Oncol 24:4783–4791. https://doi.org/10.1200/JCO.2006.06.7173
6. Tsujimoto Y, Finger LR, Yunis J et al (1984) Cloning of the chromosome breakpoint of neoplastic B cells with the t (14;18) chromosome translocation. Science 226:1097–1099
7. Kapucuoglu N, Aktepe F, Kaya H et al (2007) Immunohistochemical expression of PTEN in normal, hyperplastic and malignant endometrium and its correlation with hormone receptors, bcl-2, bax, and apoptotic index. Pathol Res Pract 203: 153–162. https://doi.org/10.1016/j.prp.2007.01.003
8. Kokawa K, Shikone T, Otani T, Nakano R (1999) Apoptosis and the expression of Bax and Bcl-2 in squamous cell carcinoma and adenocarcinoma of the uterine cervix. Cancer 85:1799–1809. https://doi.org/10.1002/(SICI)1097-0142(19990415)85:8<1799::AID-CNCR21>3.0.CO;2-M
9. Vaskivuo TE, Stenbäck F, Tapanainen JS (2002) Apoptosis and apoptosis-related factors Bcl-2, Bax, tumor necrosis factor-α, and NF-κB in human endometrial hyperplasia and carcinoma. Cancer 95:1463–1471. https://doi.org/10.1002/cncr.10876
10. Stuart ET, Gruss P (1996) PAX: developmental control genes in cell growth and differentiation. Cell Growth Differ 7:405–412
11. Stuart ET, Haffner R, Oren M, Gruss P (1995) Loss of p53 function through PAX-mediated transcriptional repression. EMBO J 14: 5638
12. Allison KH, Upson K, Reed SD et al (2012) PAX2 loss by immunohistochemistry occurs early and often in endometrial hyperplasia. Int J Gynecol Pathol 31:159–167. https://doi.org/10.1097/PGP.0b013e318226b376
13. Quick CM, Laury AR, Monte NM, Mutter GL (2012) Utility of PAX2 as a marker for diagnosis of endometrial intraepithelial neoplasia. Am J Clin Pathol 138:678–684. https://doi.org/10.1309/AJCP8OMLT7KDWLMF
14. Winyard PJ, Risdon RA, Sams VR et al (1996) The PAX2 transcription factor is expressed in cystic and hyperproliferative dysplastic epithelia in human kidney malformations. J Clin Invest 98: 451–459. https://doi.org/10.1172/JCI118811
15. Zhang LP, Shi XY, Zhao CY et al (2011) RNA interference of pax2 inhibits growth of transplanted human endometrial cancer cells in nude mice. Chin J Cancer 30:400–406
16. Mutter GL (2002) Diagnosis of premalignant endometrial disease. J Clin Pathol 55:326–331
17. Zaino RJ, Carinelli S, Ellenson LH, et al (2014) Tumours of the uterine corpus: epithelial tumours and precursors. In: Kurman RJ, Carcangiu M, Herrington C, Young R (eds) WHO Classif. Tumours female Reprod. Organs, 4th ed. WHO Press, Lyon, pp 125–126
18. Hecht JL, Ince T, Baak JP et al (2005) Prediction of endometrial carcinoma by subjective endometrial intraepithelial neoplasia diagnosis. Mod Pathol 18:324–330. https://doi.org/10.1038/modpathol.3800328
19. Popat VC, Vora DN, Gadhvi NS et al (2010) Comparison of endometrial intraepithelial neoplasia with WHO endometrial hyperplasia classification system. A comparative study of 150 cases. Histopathology 57:646–648. https://doi.org/10.1111/j.1365-2559.2010.03672.x
20. Allred DC, Harvey JM, Berardo M, Clark GM (1998) Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 11:155–168
21. Bissonnette RP, Echeverri F, Mahboubi A, Green DR (1992) Apoptotic cell death induced by c-myc is inhibited by bcl-2. Nature 359:552–554. https://doi.org/10.1038/359552a0
22. Hemann MT, Lowe SW (2006) The p53–Bcl-2 connection. Cell Death Differ 13:1256–1259. https://doi.org/10.1038/sj.cdd.4401962
23. Bozkurt KK, Yalcin Y, Erdemoglu E et al (2016) The role of immunohistochemical adrenomedullin and Bcl-2 expression in development of type-1 endometrial adenocarcinoma Adrenomedullin expression in endometrium. Pathol Res Pract 212:450–455. https://doi.org/10.1016/j.prp.2016.02.021
24. Amalinei C, Cianga C, Balan R et al (2011) Immunohistochemical analysis of steroid receptors, proliferation markers, apoptosis related molecules, and gelatinases in non-neoplastic and neoplastic endometrium. Ann Anat - Anat Anzeiger 193:43–55. https://doi.org/10.1016/j.aanat.2010.09.009
25. Wu H, Chen Y, Liang J et al (2005) Hypomethylation-linked activation of PAX2 mediates tamoxifen-stimulated endometrial carcinogenesis. Nature 438:981–987. https://doi.org/10.1038/nature04225
26. Strissel PL, Ellmann S, Loprich E et al (2008) Early aberrant insulin-like growth factor signaling in the progression to endometrial carcinoma is augmented by tamoxifen. Int J Cancer 123:2871–2879. https://doi.org/10.1002/ijc.23900
27. Kahraman K, Kiremitci S, Taskin S et al (2012) Expression pattern of PAX2 in hyperplastic and malignant endometrium. Arch Gynecol Obstet 286:173–178. https://doi.org/10.1007/s00404-012-2236-3
28. Joiner AK, Quick CM, Jeffus SK (2015) Pax2 expression in simultaneously diagnosed WHO and EIN classification systems. Int J Gynecol Pathol 34:40–46. https://doi.org/10.1097/pgp.0000000000000185