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An immunohistochemical and molecular analysis of papillary proliferation of the endometrium

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Summary

Papillary proliferations of the endometrium (PPEs) are uncommon lesions that are often associated with endometrial polyps. PPEs occasionally precede or co-exist with atypical endometrial hyperplasia or adenocarcinoma, but their pathogenesis and relationship to endometrial neoplasia is uncertain. In the present study 11 PPEs, including eight benign papillary proliferations (BPPs) and three complex papillary hyperplasias (CPHs) were examined immunohistochemically for expression of PAX2, BAF250a, p16, β-catenin and DNA mismatch repair (MMR) proteins. Molecular analysis was also performed on the CPHs using targeted next generation sequencing (NGS). All PPEs demonstrated at least one immunohistochemical abnormality with altered expression of p16 and PAX2 in nine and seven cases, respectively, and β-catenin in one case. However, none of the cases showed loss of BAF250a or MMR protein staining. All CPHs showed *KRAS* mutations with additional mutations in *AKT1* and *FBXW7* in one case each, and both *PIK3CA* and *CTNNB1* in the remaining case. Therefore, PPEs demonstrate immunophenotypical and molecular overlap with endometrial endometrioid neoplasia, although loss of BAF250a and MMR protein function do not appear to contribute significantly to these lesions. *KRAS* mutations may be important drivers in CPHs but this finding needs to be confirmed in larger studies.

Key words: Endometrium; papillary proliferation; immunohistochemistry; next generation sequencing; molecular; histopathology.

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INTRODUCTION

The presence of papillary epithelial elements in endometrial biopsy or hysterectomy specimens is often a worrisome finding since this is a common feature of endometrioid, serous and clear cell adenocarcinomas.[1] Papillary carcinomas showing squamous and/or transitional differentiation can also arise in the endometrium, although much less frequently. It is also recognised that non-malignant (hyperplastic or metaplastic) endometrial proliferations occasionally demonstrate papillary architecture and these lesions have been designated papillary proliferations of the endometrium (PPEs).[2–4] PPEs have been subdivided into simple or benign papillary proliferations (BPPs) and complex papillary hyperplasias (CPHs) based upon the degree and the extent of the architectural changes.[2] Diagnostic criteria regarding cytological atypia in PPE have varied slightly between different studies. In the largest series reported to date, Ip and colleagues specifically excluded lesions with cytological atypia although this was sometimes demonstrated in other areas of concurrent biopsies or in subsequent histological specimens.[2] However, 'mild' or 'occasional' cytological atypia has been permitted within the spectrum of PPE in other reports including the original detailed description of these lesions by Lehman and Hart.[3,4]

Irrespective of these minor variations in diagnostic criteria, there are two major practical issues related to the identification of PPEs in endometrial specimens. Most importantly, these lesions should not be misinterpreted as malignant since such a diagnosis could lead to unnecessarily aggressive treatment. Second is determining the most appropriate management of patients with PPE which in turn mainly depends upon the estimated risk of concurrent or subsequent endometrial neoplasia. This risk appears to be greater in cases of CPH than BPP, suggesting that the former should be managed in a similar manner to conventional atypical endometrial hyperplasia/endometrial intraepithelial neoplasia.[2,4]

At present the aetiology of PPE is unknown and its relationship to endometrial hyperplasia and adenocarcinoma is also uncertain. However, these lesions occasionally co-exist, suggesting that they could share pathogenetic mechanisms.[2,4] Well-established alterations in endometrial endometrioid neoplasia include *KRAS, ARID1A, PTEN* and *PIK3CA* mutations, loss of PAX2, BAF250a (ARIDIA) and DNA mismatch repair (MMR) protein expression, and activation of the Wnt signalling pathway.[5–7] The latter often results from *CTNNB1* (β-catenin) mutations and leads to the accumulation of β-catenin protein within the cytoplasm and nuclei of tumour cells contrasting with its normal cell membrane distribution. Most low-grade endometrial endometrioid adenocarcinomas show patchy ('mosaic') p16 expression, similar to normal proliferative endometrium, and this is a useful distinction in cases where usual-type (HPV-related)

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endocervical adenocarcinoma is a diagnostic consideration.[8,9] However, some subtypes of endometrial neoplasia including serous carcinoma often show diffuse p16 staining that is not HPV-related, and this is also a feature of some metaplastic endometrial alterations.[10,11]

To investigate potential pathogenetic alterations in PPEs we performed an immunohistological analysis of 11 cases (comprising eight BPPs and three CPHs), specifically targeting those changes that are commonly observed in atypical endometrial hyperplasia and adenocarcinoma. Furthermore, molecular alterations were investigated in the three more extensive CPH lesions using next-generation sequencing (NGS).

MATERIALS AND METHODS

The study group comprised 11 cases of PPE encountered by the authors between January 2012 and April 2017. Endometrial biopsies from patients on progestogen therapy, including those being managed conservatively for previously established atypical hyperplasia or adenocarcinoma were excluded. Clinicopathological details including patient age, presenting symptoms and follow-up information were obtained from the pathology reports, medical records and/or referring pathologists and clinicians. The presence of benign epithelial alterations or 'metaplasias' (tubal/ciliated, mucinous, hobnail, syncytial, eosinophilic and morular/squamous) within areas of PPE was recorded, and additional pathological changes in the endometrium were noted if relevant. The study received institutional ethics approval from King Edward Memorial Hospital.

Immunohistochemical analysis

One representative block of each case was stained immunohistochemically with the panel of antibodies summarised in Table 1 with methods as previously described.[12–14] BAF250a, PAX2 and MMR protein (MLH1, PMS2, MSH2, MSH6) expression was considered normal when the PPE demonstrated retained nuclear staining, and abnormal if there was complete loss of protein expression in the context of an adequate internal positive control. β-catenin expression was considered normal if staining was restricted to epithelial cell membranes, and abnormal if there was unequivocal nuclear staining (with or without cytoplasmic and/or cell membrane staining). Expression of p16 protein was considered abnormal if there was nucleocytoplasmic staining in >80% of cells, contrasting with the more focal (mosaic pattern) staining of normal endometrial epithelium. Additional immunohistochemical analysis for p53 and Ki-67 had been performed in eight cases (3 CPH and 5 BPP) during initial diagnostic assessment and these slides were also reviewed.

Molecular analysis (complex papillary hyperplasias)

DNA extraction

Haematoxylin and eosin (H&E) stained sections were assessed to estimate the percentage of tumour cells within the biopsy specimens. Formalin fixed, paraffin embedded tissue blocks with the highest tumour cell content were selected. The tissue fragments were transferred to Eppendorf tubes and DNA was extracted with the QIAamp DNA mini kit (Qiagen, Australia) using the Qiacube automated method. Samples for analysis with the Cobas 4800 were isolated with the Roche DNA sample preparation kit (Roche Diagnostics, Australia). DNA quantification was performed using the Nanodrop (ThermoFisher Scientific, Australia) or Qubit 2.0 Fluorometer (Life TechnologiesUSA) instruments. See Supplementary Methods (Appendix A) for further details.

Next-generation sequencing

NGS was performed using the TruSight Tumour Sequencing Panel (Illumina, USA) as previously described.[15] The genes and exons covered are summarised in Table 2. See Supplementary Methods (Appendix A) for further details.

Table 1 Summary of immunohistochemical reagents and methods

Antibody	Source	Clone	Dilution
BAF250a	Sigma-Aldrich, Australia	Polyclonal	1:200
β-catenin	Novocastra, UK	17C2	1:200
MLH1	Ventana Medical Systems, USA	M1	Predilute
MSH2	Ventana Medical Systems, USA	G219-1129	Predilute
MSH6	Ventana Medical Systems, USA	44	Predilute
PMS2	Ventana Medical Systems, USA	EPR3947	Predilute
p16	Ventana Medical Systems, USA	E6H4	Predilute
PAX2	Cell Marque, USA	EP235	1:50

RESULTS

The clinicopathological details are summarised in Table 3. The mean and median ages were 61.6 years and 65 years, respectively (range 25–85 years). Eight patients were

Table 2 Gene and exon coverage of the Trusight Tumour panel

Gene	Amplicon no.	Exons covered
AKT1	1	2
ALK	1	23
APC	14	1, 2, 3
BRAF	3	11, 15
CDH1	6	8, 9, 12
CTNNB1	2	1, 2
EGFR	7	18, 19, 20, 21
ERBB2	2	20
FBXW7	13	7, 8, 9, 10, 11
FGFR2	2	6
FOXL2	1	1
GNAQ	6	4, 5, 6
GNAS	2	6, 8
KIT	9	9, 11, 13, 17, 18
KRAS	8	1, 2, 3, 4
MAP2K1	1	2
MET	22	1, 4, 13, 15, 16, 17, 18, 19, 20
MSH6	3	5
NRAS	8	1, 2, 3, 4
PDGFRA	5	11, 13, 17
PIK3CA	15	1, 2, 7, 9, 20
PTEN	17	1, 2, 3, 4, 5, 6, 7, 8, 9
SMAD4	5	8, 11
STK11	7	1, 4, 6, 8
SRC	2	10
TP53	16	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11

Table 3 Summary of clinical and immunohistochemical findings in 11 cases of papillary proliferation of the endometrium

Case	Age	Polyp	Metaplasia	PAX2	BAF 250a	MMR protein	β-catenin	p16
CPH								
1	25	Yes	T,E,Sq	Ab	N	N	Ab	N
2	58	Yes	E,H	N	N	N	N	Ab
3	70	Yes	T,M,Sq	N	N	N	N	Ab
BPP								
4	68	Yes	T,E	Ab	N	N	N	Ab
5	52	Yes	T,E	N	N	N	N	Ab
6	57	No	T,E	N	N	N	N	Ab
7	85	Yes	T,M	Ab	N	N	N	Ab
8	57	No	E	Ab	N	N	N	Ab
9	67	Yes	E,M	Ab	N	N	N	Ab
10	65	Yes	E,H,M	Ab	N	N	N	Ab
11	74	Yes	T	Ab	N	N	N	N

Ab, abnormal; BPP, benign papillary proliferation; CPH, complex papillary hyperplasia; E, eosinophilic; H, hobnail; M, mucinous; MMR, mismatch repair; N, normal; Sq, squamous/morular; T, tubal.

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