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Case series

Using gene expression in patients with endometrial intraepithelial neoplasia to assess the risk of cancer

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ABSTRACT

Patients diagnosed with an endometrial cancer precursor lesion on biopsy may be found to have endometrial cancer at the time of subsequent surgery. The current study seeks to identify patients with endometrial intraepithelial neoplasia (EIN) on biopsy that may be harboring an occult carcinoma. Immunohistochemical stains for gene loss of expression (LOE) for 6 genes, PTEN, ARID1A, MSH6, MSH2, MLH1, and PMS2, were performed on 113 biopsy specimens with EIN. For the 95 patients with follow-up histology, 40 patients had cancer, 41 had EIN, and 14 had normal endometrium. PTEN LOE was found frequently in both EIN and endometrial cancer, and therefore had low positive predictive value. All specimens with ARID1A, MSH6, MSH2, MLH1, or PMS2 LOE on biopsy were subsequently found to have cancer. LOE of any gene was associated with modest sensitivity (0.78) in identifying patients with endometrial cancer who had EIN on biopsy. Further investigation is warranted to determine if gene LOE is a useful clinical tool when evaluating patients with EIN on biopsy.

1. Introduction

Bokhman's seminal observations in the 1980's distinguished two categories of endometrial cancer, simply classified as "type 1" and "type 2." (Bokhman, 1983) Type 1 endometrial cancer is associated with low grade endometrioid histology, and often with a hyperestrogenic phenotype, ie. obese, diabetic, and anovulatory. Type 2 cancers are generally high grade or non-endometrioid subtypes, occur in older females, and generally not associated with the typical hyperestrogenic profile. Endometrial cancer precursor lesions are typically associated with type 1 cancers. Historically, endometrial cancer precursor lesions were classified according to the 1994 World Health Organization (WHO) criteria; ie. complex atypical hyperplasia. More recently, Mutter has outlined specific objective criteria to define Endometrial Intraepithelial Neoplasia (EIN) (Mutter, 2000). WHO updated the terminology in 2014; EIN, therefore, eliminates the subjective bias of the older criteria and is generally regarded as a predecessor to malignancy.

Although EIN criteria are objective, pretreatment diagnosis of endometrial cancer precursors remain problematic. Limitations of current sampling techniques, and issues of sample quality, make it impossible to exclude the presence of coexisting carcinoma. A large Gynecologic Oncology Group trial found that approximately 40% of patients with EIN on endometrial biopsy were subsequently diagnosed with endometrial cancer on the hysterectomy specimen. (Trimble et al., 2006) Identification of patients with endometrial cancer may be important for patient counseling, treatment decisions, and planning the nature and scope of anticipated surgery. The current study, therefore, utilizes immunohistochemical (IHC) stains for specific gene loss of expression (LOE) to identify patients with EIN on biopsy who may be at higher risk of harboring an occult cancer.

2. Materials and methods

This is a retrospective single institution study performed at Queens Medical Center, Honolulu, Hawaii. All patients with EIN diagnosed on endometrial biopsy (EMB) or dilatation and curettage (D&C) from 2009 to 2014 were identified. All specimens were reviewed by a gynecologic pathologist using the criteria for EIN (Mutter, 2000). Formalin fixed tissue microarrays (TMAs) were constructed from 2.0 mm representative areas of EIN, and evaluated using IHC staining with antibodies for PTEN, ARID1A, MLH1, PMS2, MSH6, and MSH2.

Antigen retrieval was performed with EnVision FLEX Target Retrieval Solution (Dako, Santa Clara, CA) at 97-C for 20 min. Protein expression was evaluated using antibodies to MLH1 (clone ES05, 1:50

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dilution, Dako), PMS2 (clone EP51, 1:25 DILUTION, Dako), MSH2 (clone FE11,1:25 dilution, Dako), MSH6 (clone EP49,1:25 dilution, Dako), PTEN (clone 6H2.1, 1:100 dilution, Dako), and ARID1A (clone EPR13501, 1:500 dilution, Abcam, Cambridge, MA) on 4 μm tissue sections. Detection was achieved using the bond polymer refine detection kit (Leica Biosystems, Buffalo Grove, IL). Diaminobenzidine (Dako) and Hematoxylin (Dako) were used for chromogenic detection and counter staining, respectively.

The TMAs were evaluated for immunoreactivity and specimens that exhibited loss of expression (LOE) were verified with whole-slide staining. In cases of mismatch repair expression, adjacent lymphocytes and/or stromal cells were considered a positive internal control. Patients that underwent subsequent hysterectomy or repeat biopsy were identified, and all follow-up tissue was evaluated for the presence of carcinoma. Characteristics of patients were summarized by frequencies and percentages. Chi-square test or Fisher's exact test was used as a test of statistical significance; positive predictive value, negative predictive value, sensitivity, and specificity were determined with 95% confidence intervals. Analyses were conducted using SAS version 9.4 (SAS Institute, Cary, North Carolina) and p-value of less than 0.05 was considered statistically significant.

3. Results

There were 113 patients identified in the Queens Medical Center Pathology registry with a diagnosis of EIN on endometrial biopsy. Seventy-eight patients underwent subsequent hysterectomy; 17 patients received medical treatment and underwent follow-up dilatation and curettage. Eighteen patients had no follow-up tissue diagnosis, and were excluded from the analysis. For the 95 patients with follow-up histology, 40 patients were diagnosed with endometrial cancer, 41 patients had EIN, and 14 patients had normal endometrium. The results of IHC staining for PTEN, ARID1A, MLH1, PMS2, MSH2, and MSH6 and the final diagnosis of cancer versus non-cancer is summarized in Table 1. All patients with loss of ARID1A, or mismatch repair (MMR) genes MLH1, PMS2, MSH6, or MSH2 were found to have endometrial cancer on final diagnosis. Positive predictive value, negative predictive value, sensitivity, and specificity are summarized in Table 2. Of the 40 patients diagnosed with endometrial cancer on follow-up, 34 were found to have stage IA cancer. Six patients had deeply invasive or metastatic cancer. Findings for these 6 patients is summarized in Table 3. All patients with deeply invasive or metastatic cancer had at least one gene LOE.

4. Discussion

A considerable amount of data on genomic changes associated with endometrial cancer indicate that endometrial cancer is a heterogenous disease. Mutations in a variety of genes have been described. The most frequent altered pathway found in endometrial cancer is the PI3K-PTEN pathway. Mutations and absent or reduced PTEN expression have been reported in up to 80% of endometrial cancers. (Mutter et al., 2000) PTEN LOE, however, may occur early in carcinogenesis, and is noted in 55% of precancerous lesions of the endometrium (Mutter et al., 2000). The current study supports these earlier findings, with PTEN LOE a common finding in both cancer and EIN. Although PTEN LOE was found more frequently in cancer than EIN, the difference was not significant; and the positive predictive value for PTEN LOE and cancer was only 0.51.

ARID1A is key component of the chromatin remodeling complex, and is involved in the regulation of cell proliferation and differentiation. ARID1A LOE may be seen in 25% of low grade endometrial cancers and 44% of high grade endometrial cancers (Mao et al., 2013). ARID1A LOE may also be seen in 16% of endometrial cancer precursors (Werner et al., 2013). In the current study, ARID1A LOE was not seen in EIN; therefore, the specificity for identifying cancer was very high.

Defects in MMR function, including LOE of MSH6, MSH2, MLH1, and PMS2 are associated with microsatellite instability, and may be seen in approximately 40% of endometrial cancers. (The Cancer Genome Atlas Research Network, 2013) The majority of these are associated with acquired hypermethylation of the MLH1 promoter region; however MMR LOE associated with Lynch Syndrome may be seen in approximately 10% of endometrial cancers. (McMeekin et al., 2016) In the current study, all patients with MMR LOE were subsequently found to have cancer. Similar to ARID1A, MMR LOE was associated with high specificity for cancer, but low sensitivity.

Previous studies to predict cancer in EIN have focused on architectural or histopathologic markers. (McKenney and Longacre, 2009) The current study utilized IHC staining for gene LOE to identify patients at risk for cancer. A primary shortcoming of this study was that not all patients underwent definitive surgery. However, the majority of those that underwent subsequent D&C had normal endometrium on follow-up, and therefore did not require hysterectomy.

This study examines a series of patients with EIN on endometrial biopsy to determine if a panel of IHC stains for gene expression is useful in predicting the subsequent finding of cancer. ARID1A and MMR LOE were both highly correlated with the finding of cancer. PTEN LOE occurred in both cancer and EIN, and therefore had low specificity for predicting cancer. LOE of any gene had modest sensitivity (0.78) for identifying cancer; however all cases of cancer more invasive than stage IA were associated with at least one gene LOE. Although preliminary,

Table 1
Gene loss of expression and the finding of cancer.

	n (%)		p-Value
	Cancer n = 40	Non-cancer (EIN or normal) n = 55	
Loss of PTEN	24 (60%)	23 (41.82%)	0.0983
Loss of ARID1A	5 (12.5%)	0 (0%)	0.0114
Loss of MLH1, PMS2, MSH2, MSH6 (MMR)	4 (10%)	0 (0%)	0.0287
Loss of any gene	31 (77.5%)	23 (41.82%)	0.0007
Loss of ARID1A + MMR	9 (22.5%)	0 (0%)	0.0002
All normal expression	9 (22.5%)	32 (58.18%)	0.0007

Table 2
LOE for PTEN, ARID1A, MMR (MLH1, PMS2, MSH6, or MSH2), or LOE for any gene and risk of cancer. PPV = positive predictive value; NPV = negative predictive value.

LOE	PPV	NPV	Sensitivity	Specificity
PTEN	0.51 (0.36–0.66)	0.67 (0.52–0.80)	0.60 (0.43–0.75)	0.58 (0.44–0.71)
ARID1A	1.00 (0.48–1.00)	0.61 (0.50–0.71)	0.12 (0.04–0.27)	1.00 (0.94–1.00)
MMR	1.00 (0.40–1.00)	0.60 (0.50–0.71)	0.10 (0.03–0.24)	1.00 (0.94–1.00)
AIRD1A/ MMR	1.00 (0.66–1.00)	0.64 (0.53–0.74)	0.22 (0.11–0.38)	1.00 (0.94–1.00)
ANY GENE	0.57 (0.43–0.71)	0.78 (0.62–0.89)	0.78 (0.62–0.89)	0.58 (0.44–0.71)

Table 3
Patients with invasive cancer.

Patient#	Age	Grade	Stage	LOE
1	68	1	IB	PTEN
2	71	1	IB	PTEN
3	57	1	IB	ARID1a
4	59	1	IIIA	ARIDA1a
5	50	2	IIIA	MLH1, PMS2
6	37	1	IIIC	PTEN, MLH1, PMS2

these results suggest a useful role for gene LOE in pre-treatment discussions. Patients with EIN associated with abnormal gene expression should be cautioned about the possibility of invasive disease or metastases. Conversely, if normal gene expression is observed, patients may be eligible for medical management and fertility preservation, or preservation of ovarian function if hysterectomy is performed. Further investigation into utilization of gene markers as a clinical decision tool for EIN should be pursued.

Conflict of interest

The authors declare no conflict of interest.

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