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Molecular classification of endometrial carcinoma on diagnostic specimens is highly concordant with final hysterectomy: Earlier prognostic information to guide treatment

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HIGHLIGHTS

- Molecular classification can be performed on diagnostic endometrial specimens and is highly concordant with hysterectomy.
- Biologically relevant information can inform early treatment decisions, need for hereditary counselling, and stratify trials.
- Molecular classification of endometrial cancers is reproducible and identifies distinct prognostic subgroups

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ABSTRACT

Objective. Categorization and risk stratification of endometrial carcinomas is inadequate; histomorphologic assessment shows considerable interobserver variability, and risk of metastases and recurrence can only be derived after surgical staging. We have developed a Proactive Molecular Risk classification tool for Endometrial cancers (ProMisE) that identifies four distinct prognostic subgroups. Our objective was to assess whether molecular classification could be performed on diagnostic endometrial specimens obtained *prior to* surgical staging and its concordance with molecular classification performed on the subsequent hysterectomy specimen.

Methods. Sequencing of tumors for exonuclease domain mutations (EDMs) in *POLE* and immunohistochemistry for mismatch repair (MMR) proteins and p53 were applied to both pre- and post-staging archival specimens from 60 individuals to identify four molecular subgroups: MMR-D, *POLE* EDM, p53 wild type, p53 abn (abnormal). Three gynecologic subspecialty pathologists assigned histotype and grade to a subset of samples. Concordance of molecular and clinicopathologic subgroup assignments were determined, comparing biopsy/curetting to hysterectomy specimens.

Results. Complete molecular and pathologic categorization was achieved in 57 cases. Concordance metrics for pre- vs. post-staging endometrial samples categorized by ProMisE were highly favorable; average per ProMisE class sensitivity(0.9), specificity(0.96), PPV(0.9), NPV(0.96) and kappa statistic 0.86(95%CI, 0.72–0.93), indicating excellent agreement. We observed the highest level of concordance for 'p53 abn' tumors, the group associated with the worst prognosis. In contrast, grade and histotype assignment from original pathology reports pre- vs. post-staging showed only moderate levels of agreement (kappa = 0.55 and 0.44 respectively); even with subspecialty pathology review only moderate levels of agreement were observed.

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Conclusion. Molecular classification can be achieved on diagnostic endometrial samples and accurately predicts the molecular features in the final hysterectomy specimens, demonstrating concordance superior to grade and histotype. This biologically relevant information, available at initial diagnosis, has the potential to inform management (surgery, adjuvant therapy) from the earliest time point in cancer care.

1. Introduction

Endometrial carcinoma (EC) is the most common gynecologic malignancy worldwide, increasing globally in both incidence and mortality [1–4]. Histotype and grade assignment in EC is unreliable, even among expert pathologists [5–8], leading to inconsistent categorization of tumors within and between cancer centers. Current risk stratification systems used to guide adjuvant therapy are based on these irreproducible histomorphologic features. Additionally, tumor stage can only be assigned *after* definitive surgery (including hysterectomy and loss of child bearing capacity). For the approximately 14% of women diagnosed with EC under the age of 50 [9], who may be interested in fertility-sparing alternatives, this information comes too late. However all EC patients and not just these younger individuals would benefit from accurate prognostication to determine personalized treatment options (aggressiveness of surgery, chemotherapy, radiation). Our current system is inadequate; patient management, interpretation of clinical trials, and EC research have been hindered by these shortcomings.

There is a need for improved EC subgroup assignment and risk assessment. The Cancer Genome Atlas (TCGA) [10] applied array-based and sequencing methodologies on a large series of endometrioid and serous ECs, and identified four molecular subgroups of EC that were associated with differences in progression free survival. Subsequently, our group and others [11,12] have demonstrated that pared down pragmatic assays applicable in routine diagnostic practice can be used to identify four molecular subgroups. Although not identical to TCGA categorization, there is significant overlap and these subgroups are also strongly associated with outcomes. In this study we sought to determine whether our new classifier (**Pro**active **M**olecular R**is**k classification tool for **E**ndometrial cancers (**ProMisE**)) could be applied to endometrial biopsy or curetting specimens containing endometrial cancer that were obtained for diagnostic purposes, and if classification of these samples was concordant with final hysterectomy endometrial samples obtained at definitive surgical staging.

2. Methods

2.1. Cohort selection

To determine an appropriate cohort selection, an a priori power calculation was performed using the distribution of molecular subgroups in the TCGA (~7% POLE (ultramutated), 28% MSI-high, 39% CN-low and 26% CN-high), to reveal that a sample of size n = 47 would be sufficiently large to detect concordance between pre- and post-staging endometrial samples >0.65 (Power = 0.8, $\alpha = 0.05$). Previous studies [13–16] have demonstrated that it is common for grade assignment to change between diagnostic (pre-) and final (post- surgical staging) endometrial specimens ($\kappa = 0.65$); therefore, we considered the molecular classification tool (ProMisE) to be clinically useful if it improved upon this figure. In order to account for a potential loss of cases due to molecular test failure, we selected 60 women with EC where both diagnostic (pre-) and hysterectomy (post-staging) endometrial specimens were available. With Institutional Review Board approval, we identified 40 cases from our previously described EC hysterectomy cohort [11] that had undergone molecular classification with the ProMisE tool, based on the hysterectomy specimen, for whom there were available pre-surgical staging samples (endometrial biopsies or curettage specimens) that had not undergone molecular classification. These initial 40 cases were selected to ensure representation from all four molecular subgroups. We additionally identified 20 recent cases of EC where both diagnostic and final endometrial specimens were available; for these cases there was no prior knowledge of molecular subgroup. Hysterectomies performed after neoadjuvant treatment were excluded from the study to ensure that there was not disagreement between samples secondary to treatment-induced molecular changes.

The ProMisE molecular classification scheme was used to assign EC specimens (both diagnostic and final hysterectomy within the same individual) to one of four molecular subgroups using methodologies previously described [11,17]. Testing involved sequential assessment of i) IHC for MMR proteins MLH1, MSH2, MSH6 and PMS2 ii) sequencing for polymerase epsilon (*POLE*) exonuclease domain mutations (EDMs), and iii) p53 IHC (Fig. 1). Agreement of the molecular classification (ProMisE) was then compared between pre- and post-surgical staging specimens.

2.2. TMA construction

For all diagnostic endometrial samples (endometrial biopsy, endometrial curettage specimens), a tissue microarray was constructed using 0.6 mm cores in duplicate.

2.3. Immunohistochemistry

Methodological details regarding IHC for mismatch repair proteins (MLH1, MSH2, MSH6, PMS2) and for p53 have previously been described [11,18]. In cases with equivocal or uninterpretable immunohistochemical results based on the TMA slides, immunohistochemistry was repeated on full sections. Scoring was performed by one of three pathologists (CBG, QN, JL). MMR status was interpreted as lost if there was complete absence of staining in the tumor cells with adequate positive staining of internal controls (inflammatory cells or stroma). p53 was interpreted as abnormal if there was complete negative staining (null-

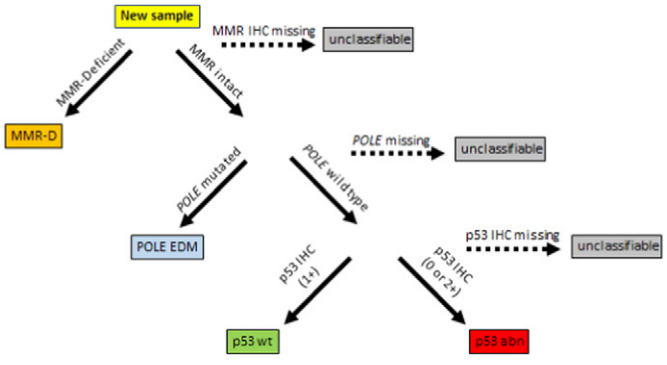

Fig. 1. New endometrial cancer samples are tested and categorized according to the above steps; First, immunohistochemistry (IHC) for the presence of mismatch repair proteins (MLH1, MSH2, MSH6, PMS2) where cases with loss of protein expression classified as MMR deficient (MMR-D). Second, sequencing for the presence of *POLE* exonuclease domain mutations (*POLE* EDM). Third, IHC for p53 to distinguish normal expression (IHC score 1) associated with wild type (p53 wt) from null/loss of function mutations (IHC score 0) or missense/gain of function mutations (IHC score 2) grouped together as p53 abn.