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An NRG Oncology/GOG study of molecular classification for risk prediction in endometrioid endometrial cancer

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HIGHLIGHTS

- Molecular classification of endometrioid endometrial cancers identifies cases at increased risk for recurrence.
- Women whose tumors have copy number alterations have reduced progression-free and cancer-specific survival.
- ~8% of endometrioid endometrial cancers are copy number altered, the majority of which are low grade, low stage cases.

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ABSTRACT

Objectives. The purpose of this study was to assess the prognostic significance of a simplified, clinically accessible classification system for endometrioid endometrial cancers combining Lynch syndrome screening and molecular risk stratification.

Methods. Tumors from NRG/GOG GOG210 were evaluated for mismatch repair defects (MSI, MMR IHC, and *MLH1* methylation), *POLE* mutations, and loss of heterozygosity. *TP53* was evaluated in a subset of cases. Tumors were assigned to four molecular classes. Relationships between molecular classes and clinicopathologic variables were assessed using contingency tests and Cox proportional methods.

Results. Molecular classification was successful for 982 tumors. Based on the NCI consensus MSI panel assessing MSI and loss of heterozygosity combined with *POLE* testing, 49% of tumors were classified copy number stable (CNS), 39% MMR deficient, 8% copy number altered (CNA) and 4% POLE mutant. Cancer-specific mortality occurred in 5% of patients with CNS tumors; 2.6% with POLE tumors; 7.6% with MMR deficient tumors and 19% with CNA tumors. The CNA group had worse progression-free (HR 2.31, 95%CI 1.53–3.49) and cancer-specific survival (HR 3.95; 95%CI 2.10–7.44). The POLE group had improved outcomes, but the differences were not statistically significant. CNA class remained significant for cancer-specific survival (HR 2.11; 95%CI 1.04–4.26) in multivariable analysis. The CNA molecular class was associated with *TP53* mutation and expression status.

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Conclusions. A simple molecular classification for endometrioid endometrial cancers that can be easily combined with Lynch syndrome screening provides important prognostic information. These findings support prospective clinical validation and further studies on the predictive value of a simplified molecular classification system.

1. Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in developed nations, with growing incidence [1]. Most cases are sporadic. An estimated 3–5% of women, however, develop EC because of an inherited mutation in DNA mismatch repair and thus have Lynch syndrome (LS). Tumor-based universal screening for LS syndrome has been recommended and adopted by many centers [2].

For most EC patients, surgery is curative. Adjuvant radiation, chemotherapy, or combined chemo-radiation therapy is used to reduce risk of recurrence in patients with clinicopathologic features that have been associated with poor outcomes. Unfortunately, currently available risk prediction algorithms for the endometrioid histologic subtype (EEC) that represents ~85% of cases are suboptimal.

Molecular classification systems to complement current approaches to risk stratification have been proposed but have not been fully validated and molecular classification for risk prediction is yet to become a standard of care. A combined tumor-based LS screening and molecular classification of risk for recurrence system could be a cost-effect approach to reducing EC disease burden. The landmark molecular profiling study of EC performed by The Cancer Genome Atlas (TCGA) included molecular classification based on whole exome sequencing, microsatellite instability (MSI) analysis, and assessment of copy number alterations across the entire genome [3]. The four molecular subtypes developed by TCGA have largely non-overlapping overall mutational burdens, distinct spectra of mutations, differences in mismatch repair (MMR as evidenced by MSI), and differences in the fraction of genome exhibiting copy number alterations. TCGA molecular subtypes are copy number high (serous-like), copy number low (endometrioid-like), POLE (ultramutated), and MSI (hypermutated).

The extensive genomic characterization undertaken by TCGA cannot be readily accomplished in most clinical settings and translation to clinical application is impractical. In 2015, Talhouk and colleagues proposed a clinically-applicable molecular-based classification system for EC [4]. A key strength of the proposed system, subsequently confirmed in a second cohort and referred to as ProMisE [5], is that analyses can be undertaken with routinely prepared pathology specimens. The p53 and MMR protein immunohistochemistry (IHC) and *POLE* mutation analyses were applied to all histologic subtypes [6]. The prognostic significance of the ProMisE in EEC, the most common subtype and the group for which decision making for use of adjuvant therapies is most challenging, however has not been fully established [7]. We sought to develop a clinically applicable molecular classification system that incorporates MMR IHC, *POLE* mutation analysis, and combined MSI and loss of heterozygosity (LOH) analysis for EEC in a large cohort developed by the Gynecologic Oncology/NRG group. The prognostic significance of molecular classification in this subtype of EC was determined and molecular class:adjuvant treatment interactions were explored.

2. Methods

2.1. Study design and participants

1040 EECs from the NRG/Gynecologic Oncology Group Study GOG210, *A Molecular Staging of Endometrial Cancer NCT00340808,* were evaluated. Subjects were accrued 2003-2007 with appropriate written consent [8]. Laboratory investigators are blinded to clinical data. Two cases studied here were also included in TCGA.

2.2. Procedures

MSI testing, IHC, and *MLH1* methylation analysis were used to classify MMR status as reported [9]. *POLE* was assessed for mutation in regions of the exonuclease domain (exons 9, 13, and 14) that harbor the majority of deleterious mutations, as described [10]. For some tumors shorter amplicons capturing the key amino acids were evaluated. Primers and conditions are provided in Table S1.

Tumors were assigned hierarchically to four molecular classes that parallel those described by TCGA [3]: copy number altered (CNA), copy number stable (CNS), POLE mutant, and MMR deficient (Fig. S1). Tumors were classified MMR deficient based on MSI and/or IHC defects. For cases with normal MMR, determination of copy number status was based on LOH at three highly informative microsatellite repeats included in the 5-plex MSI analysis [11]. Fragments sizes and peak heights were used to determine informativity and if there was evidence of loss of heterozygosity (LOH), no loss (NL), or not informative (homozygous). LOH at one or more marker was used to classify a tumor as CNA. Tumors informative for at least one marker and retained heterozygosity were classified as CNS. The POLE class was assigned to CNS cases (CNA and MMR deficient tumors with *POLE* mutations were not classified as POLE).

2.2.1. Analysis of TP53

TP53 mutation testing was performed for 20 tumors from each molecular class. Laboratory investigators are blinded to the clinicopathologic data and there was no selection for any feature other than molecular class. The entire coding region (exons 2-11) was evaluated using PCR amplification and Sanger sequencing (Table S1). For all *TP53* mutations, the relative peak heights for somatic variants were used to determine if there was loss of the wild-type allele (LOH). Single nucleotide polymorphisms (SNPs) were also used to assess LOH. For *TP53* variants not previously reported as mutations or when peak height for the normal and variant bases were similar, corresponding normal DNAs were tested to confirm mutations were somatic.

2.2.2. p53 Immunohistochemistry

IHC for p53 was performed on formalin-fixed paraffin-embedded tissue sections using a mouse monoclonal anti-human antibody (Novocastra™ Clone DO-7) at a dilution of 1:800. Staining was performed on a representative whole section using the Leica Bond RX autostainer (Leica Biosystems, Buffalo Grove, IL, USA). Heat induced epitope retrieval was performed using ER2 antigen retrieval solution. Stained slides were examined by an experienced gynecologic pathologist [AAS] who was blinded to molecular data. Results were reported as "wild-type" or "mutant" pattern. A "mutant" pattern was defined as strong nuclear staining in >60% of neoplastic cells with adequate internal controls.

2.3. Outcomes

The primary endpoints were progression-free and endometrial cancer-specific survival for the four molecular classes of EEC with hazard ratios and confidence intervals.

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