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Assessing the prognostic role of *ATR* mutation in endometrioid endometrial cancer: An NRG Oncology/Gynecologic Oncology Group study

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HIGHLIGHTS

- *ATR* is a mutational target in tumors with defective DNA mismatch repair.
- We could not validate the prognostic significance of *ATR* mutations in endometrioid tumors.

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ABSTRACT

Objective. We sought to validate the clinicopathologic implications and prognostic significance of *ATR* (ataxia telangiectasia mutated and Rad3-related) mutation in patients with endometrioid endometrial cancer and defective DNA mismatch repair enrolled in a cooperative group molecular staging study of endometrial cancer.

Methods. After pathology review, only endometrioid tumors with high neoplastic cellularity (≥70%) and high quality DNA for molecular analyses were included. MSI (microsatellite instability) typing was performed and the target sequence in exon 10 of *ATR* was evaluated by direct sequencing in all MSI-high tumors. Associations between *ATR* mutations and clinicopathologic variables were assessed using contingency table tests. Differences in overall survival (OS) and disease-free survival (DFS) were evaluated by univariate analyses and multivariable Cox proportional hazard models.

Results. A total of 475 eligible cases were identified. Of 368 MSI+ cases, the sequence of interest could be successfully genotyped in 357 cases. *ATR* mutations were exclusively identified in 46 tumors with high level microsatellite instability (MSI+) (12.9%, $p < 0.001$) and were associated with higher tumor grade ($p = 0.001$). *ATR* mutations were not associated with OS (HR 1.16; 95% CI, 0.58–2.32; $p = 0.68$) or DFS (HR 0.61; 95% CI, 0.25–1.50; $p = 0.28$).

Conclusion. Truncating mutations in exon 10 of *ATR* occur exclusively in tumors with evidence of defective DNA mismatch repair. We were not able to confirm the prognostic value of these mutations in patients with endometrioid endometrial cancer.

1. Introduction

Endometrial cancer is the fourth most common malignancy affecting American women. The incidence and mortality associated with this

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disease have increased over the last decade [1]. Despite an initially anticipated good prognosis, some patients will present with advanced stages or experience disease recurrence or progression. Several clinicopathologic models have been proposed to identify patients at risk of recurrence and death from endometrial cancer. These strategies have the ultimate objective of identifying individuals who would most benefit from postoperative therapeutic interventions.

The clinical utility of various clinical, surgical and pathologic risk assessment models for patients with endometrial cancer remains suboptimal. Therefore, attention is being aimed at identifying molecular signatures that could predict clinical outcomes and potentially guide the development of targeted therapies. Multiple molecular alterations have been described in the histogenesis and progression of endometrial cancer. Traditionally, PTEN loss, defects in DNA mismatch repair as well as mutations in KRAS2, CTNNB1, RB, and TP53 appeared characteristic in endometrioid tumors. Recent work has demonstrated that beyond histologic types, it is possible to categorize endometrial cancers into four categories: POLE ultramutated (POLE codes for the central catalytic subunit of DNA polymerase epsilon), copy-number low, copy-number high and interestingly a group of microsatellite instability hypermutated tumors [2,3].

An estimated 10–30% of endometrial cancers exhibit microsatellite instability (MSI), a quantifiable phenotype of tumors with deficient DNA mismatch repair [3–8]. It has been proposed that tumors with defective DNA mismatch repair accumulate deleterious mutations. ATR is a serine/threonine-specific protein kinase that is involved in sensing DNA damage and activating the DNA damage checkpoint, leading to cell cycle arrest. Somatic mutations have been identified in exon 10 of *ATR* in endometrioid endometrial tumors with DNA mismatch repair defects [9,10]. These insertion/deletion variants involve the A10 mononucleotide of exon 10 of ATR and result in early stop codons. The truncate ATR product has been shown to provide a survival advantage to cancer cells. These mutations in exon 10 of ATR are independent prognostic markers of disease-free and overall survival among patients with endometrioid endometrial cancer [11,12].

We sought to validate the clinicopathologic implications and prognostic significance of *ATR* mutation in patients with endometrioid endometrial cancer and defective DNA mismatch repair enrolled in a cooperative group molecular staging study of endometrial cancer.

2. Methods

2.1. Study participants and clinical data

The objectives and specifics of Gynecologic Oncology Group's GOG0210: a molecular staging study of endometrial carcinoma (NCT00340808) have been previously reported [13,14]. Briefly, women undergoing surgical staging of newly diagnosed endometrial cancer were enrolled in this protocol. The study was approved by the institutional review boards of all participating institutions and all patients consented for participation. Clinical data, tumor, and biospecimens (e.g. blood and urine) for biomarker research were collected at the time of surgery. Eligibility, clinical reports and pathology was centrally reviewed by NRG/GOG for each case. A total of 3838 subjects were enrolled between September 2003 and September 2007 (when enrollment criteria were restricted). Of those, 2715 evaluable cases had endometrioid endometrial tumors and were evaluated by the GOG Tissue Bank for potential inclusion in the present study.

Only cases of histologically confirmed endometrioid endometrial adenocarcinoma with viable tissue and high quality DNA available were included in the present study (N = 475). Despite initial assumptions anticipating a high yield among these endometrioid endometrial cases, upon tissue bank pathology review for the present and other GOG-210 related studies, it was noted that unfortunately less than 60% of endometrioid endometrial adenocarcinomas had high neoplastic cellularity (≥70%) tissue available for molecular analyses.

2.2. Tissue processing, MSI typing, and ATR genotyping

DNA was extracted in a semi-automated fashion using a Maxwell 16 nucleic acid purification system (Promega Corporation; Madison, WI). DNA concentration and purity was measured by nanodrop spectrophotometry.

MSI typing was performed as previously described using five National Cancer Institute consensus microsatellite markers (BAT25, BAT26, D2S123, D5S346, and D17S250) [15]. Multiplex analysis of PCR products generated using fluorescent primers relied on ABI 3130 genetic analyzer and GeneMapper software v4.0 (Applied Biosystems Inc; Foster City, CA). Standard definitions for MSI were applied as previously described [12,15]. Cases were designated as MSI-high (MSI+) if novel PCR bands were present in at least two of the five consensus panel markers. Cases were designated as MSI-low if a novel PCR band was identified in at least one of the five consensus panel markers and as microsatellite stable if there was no evidence of MSI in any of the five markers.

DNA aliquots of all tumors with evidence of MSI underwent *ATR* mutation analysis. The A10 mononucleotide repeat of exon 10 of *ATR* (#ENSG00000175054) was amplified by PCR (433-bp amplicon) using the Deep VentR high-fidelity DNA polymerase (New England Biolab, Ipswich, MA) with the following primers: 5′-CACGGCATGTTTTATCTG ACA-3′ (forward) and 5′-TCAGGTATGCCCCATTTAGG-3′ (reverse) [Tm = 63 °C]. Amplification products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA) and sequenced unidirectionally with the ABI Prism BigDye Terminator chemistry version 1.1 (Applied Biosystems, Foster City, CA). Sequencing was carried out at the Washington University School of Medicine's Protein and Nucleic Acid Chemistry Laboratory.

All *ATR* sequences were analyzed using Sequencher DNA analysis software (v4.9; Ann Arbor, MI) and putative insertion/deletion variants involving the A10 mononucleotide of interest in exon 10 of ATR were visually inspected to confirm the presence of mutations. Ambiguous sequence reads were resolved by repeat sequencing reaction and/or repeat PCR to generate sequencing template. Non-informative specimens were analyzed at least three times.

2.3. Statistical analyses

The primary objective of this study was to validate the clinicopathologic associations and prognostic significance of *ATR* mutation in endometrioid endometrial cancer cases with defective DNA mismatch repair.

The relationship between *ATR* mutation status and covariates was assessed using Chi-square test, Fisher's exact test or Student's t-test as appropriate. Overall survival (OS) was defined as the time (in months) from study enrollment to death due to any cause. Disease-free survival (DFS) was defined as the time from date of enrollment to date of recurrence, progression or death due to disease. Survivors were censored at the date of last contact. Relative to DFS, patients who did not die of disease were censored at the date of death. The Kaplan–Meier product limit method was used to estimate OS and DFS. Differences in OS and DFS by *ATR* mutation status were evaluated using the log-rank test. Univariate and multivariate Cox proportional hazard regression models were fitted to assess the effects of known covariates and *ATR* mutation status on OS and DFS. Covariates that were significant on univariate analysis (p-value < 0.2) were included in the corresponding multivariate model after adjusting for known prognostic factors. All analyses were two-sided and significance was set at a p-value of 0.05. Statistical analyses were performed using either SAS (Cary, NC) versions 9.2 or R.

Previous studies proposed *ATR* mutation to be an independent prognostic variable for both OS (hazard ratio [HR], 3.88; 95% confidence interval [CI], 1.64 to 9.18; p = 0.002) and DFS (HR, 4.29; 95% CI, 1.48 to 12.45; p = 0.007) [12]. Assuming proportional hazard rates and previously observed differences attributable to *ATR* mutations and