High-Intermediate Risk Endometrial Cancer: Can Gene Expression Predict Recurrence?
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Introduction
• Endometrial cancer (EMCA) is the most common gynecologic cancer in the United States.
• Most cases are diagnosed at Stage 1. and have excellent prognosis after surgery alone.
• A subset of early-stage endometrial cancer patients are at higher risk for recurrence.
• Prognostic factors of recurrence of early stage EMCA include lymphovascular space invasion (LVI), histologic grade (2 or 3), depth of myometrial invasion (>50%), and patient age.
• Results from GOG 99 and PORTEC-2 led to the designation of a subset of patients known as high-intermediate risk (H-IR) EMCA based on reproducible pathologic risk factors.
• H-IR EMCA patients are at 20-30% risk of recurrence even in the setting of early stage disease.
• Studies have shown that adjuvant therapies increases progression free survival, but does not affect overall survival in these patients.

Objective: To identify a gene signature that determines which H-IR EMCA patients are at the highest risk for recurrence and to identify RNA expression changes that occur in the recurrent tumor.

Methods
• IRB-approved retrospective cohort study.
• All patients who underwent surgery at UAB and met criteria for H-IR EMCA based on GOG 99 between 2000-2010.
• Clinical data was collected on all patients, but only patients who did not receive adjuvant treatment were included in this analysis.
• FFPE slides were made from patients that recurred and an equal number of patients that did not recur were matched on a case-by-case basis (controls).
• Tissue was available for analysis from 5 patients at the time of recurrence.

DNA-CARIS protocol: NextSeq, a custom-designed SureSelect XT assay (592 whole-gene targets) was performed on 26 archival FFPE tumors (13 that recurred, 13 control). All variants were detected with >99% confidence based on allele frequency and average coverage of >5X0 and an analytic sensitivity of 5%. Tumor DNA was harvested by manual microdissection. Genes variants identified were interpreted by board-certified molecular geneticists and categorized based on ACMG standards.

RNA-Nanostar protocol: Gene expression data was collected for 770 genes using the Nanostar nCounter PanCancer Pathway Panel on 26 primary archival FFPE tumors (13 that recurred, 13 control) and on tumor from 5 of the 13 pts that recurred at the time of recurrence. Molecular profiles and pathway analysis of the cohorts (recurred vs. did not; primary vs. recurrent) were compared using qPath panel Analysis Software. Genes were evaluated using a fold change of 2 and a p-value of <= 0.05.

Table 1 and 2: Matched Patient Demographics

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<th>Variable</th>
<th>Diagnosed</th>
<th>Recurred</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Age of Diagnosis</td>
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<td>61.67</td>
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<td>Stage</td>
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<tr>
<td>Recurrence</td>
<td>32.62</td>
<td>26.20</td>
<td>0.43</td>
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Table 1: Patient Demographics

Figure 1. Characteristics of DNA mutations in EMCA patients that Recurred and did NOT Recurred. A. Average number of total mutations found in matched groups of H-IR EMCA patients. B. Top 10 genes in all patients. C. Top 10 genes in Patients that did NOT Recurred. D. Top 10 genes in Patients that Recurred. E. Volcano plot of genes differently expressed in tissue at time of recurrence. F. Table of top few genes that are significantly altered at recurrence based on log fold change.

Figure 2. A. Differences in 13 pathway scores of patients that did not recur vs. patients that recurred. B. Plots of individual patient pathway scores in H-IR EMCA-Recurred. C. Expression Analysis. D. Volcano plot of genes differentially expressed in patients that recurred did not recur. E. Table of top 5 genes that are significantly different based on log fold change in those that recurred.

Figure 3. A. RNA expression pathway analysis of patients that recurred vs. matched patients that did not recur. Two pathways were significantly up regulated in the patients that did recur: Cell Cycle-Apoptosis and DNA Damage-Repair.

Figure 4. List of DNA mutations in primary tumor at the time of diagnosis in 4 H-IR EMCA patients that recurred and RNA expression pathway analysis in primary tumor compared to recurrent tumor.

Conclusions
• Similar number and type of DNA mutations were found in patients that recurred compared to matching patients that did not recur.
• MSI-high and mutations in AKT1, DICER1, BRD3, PIK3CA, PD04609, RNF213, and BCOR genes were more commonly observed in patients that recurred.
• DNA expression pathway analysis of EMCA patients that recurred vs. matched patients that did not recur resulted in 2 pathways that were significantly up regulated in the patients that recurred: Cell Cycle and DNA Damage.
• Nine of 13 pathways were significantly altered in tissue samples from the time of recurrence compared to tissue from the time of diagnosis.
The results of this study are hypothesis generating and will need to be validated in a much larger cohort.
• These findings could potentially impact the decision to treat in H-IR EMCA patients with adjuvant therapy.

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