Methylation Makers for Detection of Endometrial Carcinoma

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Endometrial cancer survival

- Good prognosis when detected early, but 25% of cancers present at advanced stage and have poor survival
- About 80% of endometrial cancer deaths occur due to advanced stage type 1 cancers

Adenocarcinoma of the Corpus Uteri:
Relative Survival Rate (%) by AJCC Stage (SEER modified 3rd edition), Ages 20+, 12 SEER Areas, 1988-2001
Promise of early detection

- Most common gynecological cancer in the US: 49,560 cases and 8,190 deaths in 2013
- **Well defined high risk populations:** Women with high BMI, postmenopausal bleeding, endometrial hyperplasia
- 1-2 million office visits each year in the US related to postmenopausal bleeding
- No uniform management of women at increased risk of endometrial cancer
Methylation profiling of endometrial cancers

**Discovery**
- Polish Endometrial Cancer Case-Control Study: 148 cancers
- Benign Reproductive Tissue Study I: 25 normal tissues

**Replication**
- Endometrial Hyperplasia Study: 69 cancers
- Benign Reproductive Tissue Study II: 43 normal tissues

- Endometrial tissues from three population-based studies
- DNA was extracted from paraffin-embedded tissue
- Methylation analysis on Illumina Golden Gate platform, covering >800 cancer related genes
- Finding markers of etiologic heterogeneity and for early detection
Methylation patterns show etiologic heterogeneity

- **Two major cancer clusters:** One cluster with high prevalence of microsatellite instability (MSI)
- **Comparison of cancer and normal tissue:** Over 300 sites with $p<0.001$; PTEN pathway was most significantly methylated
Genes with different methylation levels between endometrial cancer and normal endometrial tissue

<table>
<thead>
<tr>
<th>Gene</th>
<th>control</th>
<th>case</th>
<th>difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCL2</td>
<td>0.12</td>
<td>0.78</td>
<td>0.66</td>
<td>&lt; 10^-7</td>
</tr>
<tr>
<td>HTR1B</td>
<td>0.10</td>
<td>0.75</td>
<td>0.65</td>
<td>&lt; 10^-7</td>
</tr>
<tr>
<td>HS3ST2</td>
<td>0.19</td>
<td>0.78</td>
<td>0.59</td>
<td>&lt; 10^-7</td>
</tr>
<tr>
<td>SOX1</td>
<td>0.20</td>
<td>0.76</td>
<td>0.56</td>
<td>&lt; 10^-7</td>
</tr>
<tr>
<td>MME</td>
<td>0.06</td>
<td>0.61</td>
<td>0.55</td>
<td>&lt; 10^-7</td>
</tr>
<tr>
<td>ADCYAP1</td>
<td>0.09</td>
<td>0.61</td>
<td>0.52</td>
<td>&lt; 10^-7</td>
</tr>
<tr>
<td>NPY</td>
<td>0.17</td>
<td>0.68</td>
<td>0.51</td>
<td>&lt; 10^-7</td>
</tr>
<tr>
<td>CDH13</td>
<td>0.14</td>
<td>0.54</td>
<td>0.40</td>
<td>1.8x10^-6</td>
</tr>
</tbody>
</table>

- Top eight candidate genes with high methylation differences, low methylation in controls (probes averaged for each gene)

Wentzensen et al. IJC in press
Replication of eight candidate genes in the validation study
Prediction of endometrial carcinomas using methylation markers

- Two classifiers based on (1) top 8 genes and (2) all 800 genes included on the array were successfully replicated in independent samples.
## Replication of methylation markers in TCGA

<table>
<thead>
<tr>
<th>Gene</th>
<th>Normal tissues (n=43)</th>
<th>Endometrioid Carcinomas</th>
<th>Serous Carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cases (n=358)</td>
<td>p-value</td>
</tr>
<tr>
<td>ADCYAP1</td>
<td>0.07</td>
<td>0.60</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ASCL2</td>
<td>0.12</td>
<td>0.55</td>
<td>0.0001</td>
</tr>
<tr>
<td>CDH13</td>
<td>0.16</td>
<td>0.54</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HS3ST2</td>
<td>0.12</td>
<td>0.55</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HTR1B</td>
<td>0.12</td>
<td>0.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MME</td>
<td>0.20</td>
<td>0.48</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NPY</td>
<td>0.10</td>
<td>0.57</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SOX1</td>
<td>0.14</td>
<td>0.60</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

- All eight markers replicate in endometrioid cancers from TCGA
- Four (to five) markers replicate in serous cancers from TCGA
Evaluation of candidate markers in lower genital tract samples

1. **Tao brush**: Sampling from complete uterine epithelium

2. **Tampon**: Collecting blood and discharge from the vaginal pool

- **Pilot study with Mayo Clinic**: Collection of lower genital tract samples from 40 women with cancer and 40 women without cancer
Replication of candidates in lower genital tract specimens

- 5/5 candidates were successfully replicated in Tao brush samples
- 4/5 candidates were successfully replicated in Tampon samples

Wentzensen et al. IJC in press; Bakkum-Gamez, Wentzensen et al. submitted
NPY methylation in cases and controls

- Increasing dilution of methylation signal
- Good discrimination of case-control status
Performance of markers in Tao brush samples

![ROC curve showing performance of markers in Tao brush samples](image)
Performance of the combined analysis of 11 CpG sites in Tampon samples

- The AUC of the combined model is 0.85
- At a cutoff of 1 or more hypermethylated sites, the assay has 83% sensitivity and 83% specificity
How can the risk stratification of methylation markers be used clinically?

• Refer women to treatment
• Refer women for further diagnostic evaluation
• Reassure women that no further evaluation is necessary
A study to evaluate methylation markers for detection of endometrial carcinoma

- 1,000 women 45 years or older presenting at Mayo Clinic
  - Evaluation of abnormal endometrial bleeding, discharge, thickening of endometrial stripe
- At least 5% estimated prevalence for atypical hyperplasia as well as cancer
- Collection of Tampon and Tao brush samples, blood, tissue from endometrial biopsies and surgery, RF data
- 2-year follow-up of women without endometrial cancer
Risk stratification for prediction of endometrial cancer

- Possibility of self-sampling
- Integration of clinical symptoms and methylation markers into risk prediction models
- Evaluation of methylation markers in endometrial hyperplasia

Wentzensen and Wacholder Cancer Discovery 2013
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