Identification of an exosomal-derived biomarker signature for early detection of ovarian cancer in BRCA mutation carriers

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I have no financial relationships with ACCME defined ineligible companies to report.

I will not be discussing any unlabeled or investigational uses of any pharmaceutical products or medical devices.
No Current Screening Tool for Ovarian Cancer
Increased Risk of Ovarian Malignancy with BRCA Mutation

King et al. Science 2013

### Cumulative Ovarian Cancer Incidence vs Age

- **BRCA1**
- **BRCA2**

<table>
<thead>
<tr>
<th>Screening Method</th>
<th>Frequency of Screening</th>
<th>Surgical Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine Screening</td>
<td>Starting at age 30-35, or 5-10 years earlier than family member’s age at time of diagnosis</td>
<td>RRSO at age 35-40 for BRCA1, at age 40-45 for BRCA2</td>
</tr>
<tr>
<td>TVUS or CA 125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACOG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **SGO**
  - Routine screening generally not recommended. Short term surveillance until RRSO is reasonable
  - Starting at age 30-35, or 5-10 years earlier than family member’s age at time of diagnosis
  - RRSO at age 35-40 for BRCA1, at age 40-45 for BRCA2
  - TVUS or CA 125

- **NCCN**
  - Routine screening not recommended
  - Case-by-case basis beginning at age 30-35
  - RRSO at age 35-40 or at completion of childbearing. May delay until age 40-45 for BRCA2 if patient has had a bilateral mastectomy
  - TVUS or CA 125

- **USPSTF**
  - No recommendations in high risk population

Lewis et al. Cancers 2018
Potential Biomarkers for Ovarian Cancer Screening

- Risk of Ovarian Cancer Algorithm

- Serum tumor markers:
  - CA 125
  - HE4

- Autoantibodies

- Serum miRNA

- ctDNA


Exosomes and Cancer

Cao, Cun et al. unpublished
The Utility of Exosomes in Cancer Biology

Yu et al. Molecular Cancer 2022
Objective

To identify a distinct signature of exosomal small noncoding RNAs to serve as a biomarker for detecting early-stage ovarian carcinoma in patients with BRCA mutations
Methods
Patient Selection and Exosomal RNA sequencing

500ul Pre-operative plasma from 30 *BRCA1* or *BRCA2* mutation carriers with ovarian cancer (Cases)

Exosomal RNA isolation

RNAseq

Identification of differentially expressed exosomal miRNA

500ul Pre-operative plasma from 30 age-matched *BRCA1* or *BRCA* mutation carriers without ovarian cancer (Controls)

Exosomal RNA isolation

RNAseq
Analysis and Modeling Pipeline

- Fastq file
- Alignment
- Differential Expression Analysis
- Machine learning modeling
- Ensemble modeling
- Remove Adapters
- Quality Control
- Feature Engineering
- Training/testing set
- Investigate early-stage samples
Results
# Clinical and Demographic Characteristics of Patients

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median age (year)</strong></td>
<td>49.7</td>
<td>49.5</td>
</tr>
<tr>
<td><strong>Mutation (n)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>BRCA2</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>BRCA1 and 2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Race (n)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Hispanic</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Black</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Asian</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Median CA 125 (units/ml)</strong></td>
<td>1387</td>
<td>10</td>
</tr>
<tr>
<td><strong>Stage at Diagnosis (n)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STIC</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Stage I</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Stage II</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td>16</td>
<td></td>
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<tr>
<td>Stage IV</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Histology (n)</strong></td>
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<td></td>
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<tr>
<td>Serous</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>2</td>
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</table>

Early Stage Samples: STIC, Stage I, Stage II
Heterogenous Expression of Exosomal RNA

Summary of Reads Mapping to Annotation Types

- Antisense to exons
- Antisense to introns
- Antisense to ncRNAs
- Antisense to repeat elements
- CDBox
- HAcBox
- LINE
- LTR
- Other Rfam ncRNA
- RefSeq exons
- RefSeq introns
- RefSeq IncRNA
- SINE
- Tandem repeat
- Unannotated
- lincRNA
- miRNA
- piRNA
- rRNA
- scaRNA
- IRNA
- IRNA_like
Differences in Non-Coding RNA Expression

P <0.05
Fold change >1 or <-1

Color Key and Histogram

patient sample
Model Generation

Training set

Testing set
## Top 10 Candidates

<table>
<thead>
<tr>
<th>hsa-mir-203a</th>
<th>tRNA-Ala-GCY_copy6</th>
<th>HBII-336</th>
<th>mgU2-19-30</th>
<th>linc-UNC13C-2-antisense</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-548a-3p</td>
<td>IER3-copy61</td>
<td>linc-FOXA2-1-copu2-antisense</td>
<td>hsa-miR-539-3p</td>
<td>LSU-rRNA_Hsa_copy15</td>
</tr>
</tbody>
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## Performance of Signature Biomarker

<table>
<thead>
<tr>
<th>All Stage Assessment</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=20 Signature Biomarker Panel</td>
<td>0.7</td>
<td>1</td>
<td>0.85</td>
<td>1</td>
<td>0.77</td>
</tr>
<tr>
<td>CA 125 alone</td>
<td>0.8</td>
<td>1</td>
<td>0.9</td>
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</tr>
<tr>
<td>Signature Biomarker Panel</td>
<td>0.82</td>
<td>1</td>
<td>0.95</td>
<td>1</td>
<td>0.94</td>
</tr>
<tr>
<td>CA 125 alone</td>
<td>0.55</td>
<td>1</td>
<td>0.88</td>
<td>1</td>
<td>0.86</td>
</tr>
</tbody>
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Conclusions
Conclusions

Limitations

• Small patient population

Future Directions

• Need for larger study for validation
• Development of potential commercial assays
• Continued advancements in machine learning and cancer biology
Conclusions

A signature of exosomal noncoding-RNA that is differentially expressed that when combined with CA 125 levels provides a biomarker model that improved the detection of early-stage ovarian cancer in BRCA1 and 2 mutation compared to CA 125 levels alone.
References


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