Assessing inter-component heterogeneity of biphasic uterine carcinosarcoma
Research Grand Rounds

Brief Outline:

a) Introduce the Avera Institute for Human Genetics

b) Highlight a Recent Publication by Avera

c) Example of Research Integration at Avera
Avera Institute for Human Genetics

- State of the art Genetics Laboratory - 16,000 ft² (~ 1500 m²)
- A part of an Integrated Health System (Avera Health)
- CLIA/CAP Certified
- 4 Laboratory Suites
  - Sample Accessioning
  - DNA Extraction
  - Pre-PCR
  - Analysis
- Multidisciplinary Team
Integration of Personalized Medicine Initiatives within our clinical practices across our Avera Health System ....and beyond

Advance science to further understand the contribution of hereditary predisposition to personality, growth, development, disease and risk factors for disease and translate this knowledge to transform health care
Sample Accessioning

Sample tracking Software
• BSI Systems (Biological Specimen Inventory System)

Sample Storage
• 2D Matrix Tubes and Racks
• Walk-in Refrigerator 4°C
• Walk-in Freezer -20°C
DNA Extraction Laboratory

- Various Tissue Sources
  - Blood, Buccal, Frozen Tissue, FFPE, Fecal
- DNA / RNA
- Manual and Automated
Pre-PCR Laboratory

- DNA/RNA Quantitation
  - Quality Measurements
- PCR Set-up
  - Hamilton Nimbus
  - Qiagen QIAgility
- DNA Shearing (NGS)
  - Sonication
## Analysis Laboratory

**Largest Laboratory Suite**
- Illumina iScan, Tecan Robot, Microarray Autoloader
- HiSeq 2500, cBot
- MiSeq
- ABI ViiA7 and QuantStudio 7 Real-Time PCR Machines
- Agilent 2100 BioAnalyzers
- BioRad Imager
Next Generation Sequencing

HiSeq 2500

Whole Genome Sequencing
Whole Exome Sequencing
Whole Transcriptome
ChIP Sequencing
Bisulfite Sequencing
miRNA Sequencing

MiSeq

Targeted Sequencing
Cancer Panels
Custom Panels
Microbiome 16s rRNA
Question: What are the molecular features of the separated tumor components of uterine carcinosarcoma?
Uterine Carcinosarcoma (UCS)

- Cancer that develops in the uterus
- Tumor displays histological features of both endometrial carcinoma and sarcoma
- Rare and aggressive form cancer (< 5% of all uterine cancers)
- In the US, about two per 100,000 women will develop UCS annually
- Approximately only 35% of patients survive 5-years after diagnosis
  - < 10% 5-year survival for late-stage diagnosis
  - Aggressive clinical course
  - Disproportionate number of all uterine cancer deaths (15%)

- Also known as malignant mixed mullerian tumor
- Features
  - Biphasic Tumor
    - Epithelial - Malignant glandular component (adenocarcinoma)
    - Mesenchymal - Malignant stromal component (leiomyosarcoma, fibrosarcoma, rhabdomyosarcoma, chondrosarcoma, and osteosarcoma)
Tumor Heterogeneity

HapLOH – Developed by Paul Scheet at MD Anderson Cancer Center (2014)

Can detect heterogeneity down to 2% or lower of tumor cells in population
Uterine Carcinosarcoma (UCS)

Objective:
Study the genomic and transcriptomic profiles of the separated biphasic elements to gain further insights into the development of these tumors

Hypothesis:
UCS develops from a common stem cell in a monoclonal fashion
Methods

- **Research Participants**
  - Avera Patients (Drs. Rojas, Starks, and Sulaiman)
  - Consented to Gynecological Specimen Bank research protocol (#2015.020)
  - 10 patients with homologous UCS

- **Specimens**
  - FFPE Tumor Tissue Block
  - Macro-dissection from same block
    - Carcinoma
    - Sarcoma
    - Adjacent normal tissue

Patient clinical overview.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age at diagnosis (years)</th>
<th>Stage at diagnosis</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0317</td>
<td>58</td>
<td>IIB</td>
<td>Complete response</td>
</tr>
<tr>
<td>0609</td>
<td>89</td>
<td>IIA</td>
<td>Complete response</td>
</tr>
<tr>
<td>0619</td>
<td>64</td>
<td>IB</td>
<td>Complete response</td>
</tr>
<tr>
<td>0622</td>
<td>68</td>
<td>IIA</td>
<td>Complete response</td>
</tr>
<tr>
<td>0724</td>
<td>39</td>
<td>IVB</td>
<td>Progressive disease</td>
</tr>
<tr>
<td>0819</td>
<td>71</td>
<td>IIB</td>
<td>NA</td>
</tr>
<tr>
<td>0829</td>
<td>61</td>
<td>IA</td>
<td>Complete response</td>
</tr>
<tr>
<td>0909</td>
<td>65</td>
<td>IA</td>
<td>Complete response</td>
</tr>
<tr>
<td>1104</td>
<td>74</td>
<td>IIIA</td>
<td>Complete response</td>
</tr>
</tbody>
</table>
Methods

- All homologous Cancers
  - Heterologous tumors are made up of tissue differing from that in which it grows
- Reduce a source of variation

<table>
<thead>
<tr>
<th>Subject</th>
<th>Carcinoma</th>
<th>Sarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>0317</td>
<td>Adenocarcinoma NOS</td>
<td>Leiomyosarcoma, mostly Sarcomatous Component</td>
</tr>
<tr>
<td>0609</td>
<td>Adenocarcinoma, Endometrioid Grade III</td>
<td>Leiomyosarcoma</td>
</tr>
<tr>
<td>0619</td>
<td>Adenocarcinoma, Endometrioid Grade I-II; No High Grade Carcinoma Components</td>
<td>Leiomyosarcoma</td>
</tr>
<tr>
<td>0622</td>
<td>Adeno-squamous Carcinoma</td>
<td>Leiomyosarcoma</td>
</tr>
<tr>
<td>0724</td>
<td>A Small Component of Well-Differentiated Endometrioid Adenocarcinoma Grade I</td>
<td>Leiomyosarcoma</td>
</tr>
<tr>
<td>0819</td>
<td>High Grade Adenocarcinoma NOS</td>
<td>Leiomyosarcoma</td>
</tr>
<tr>
<td>0829</td>
<td>Adenocarcinoma, Endometrioid Grade III</td>
<td>Small Component of Leiomyosarcoma less than 5%</td>
</tr>
<tr>
<td>0909</td>
<td>Adenocarcinoma NOS</td>
<td>Leiomyosarcoma</td>
</tr>
<tr>
<td>1104</td>
<td>A Small component Adenocarcinoma NOS</td>
<td>Mostly Leiomyosarcoma</td>
</tr>
<tr>
<td>0115</td>
<td>Poorly Differentiated Adenocarcinoma Grade III</td>
<td>Leiomyosarcoma</td>
</tr>
</tbody>
</table>
Methods

DNA & RNA Extraction (Qiagen AllPrep DNA/RNA FFPE Kit)

Whole Genome SNP Genotyping
- Illumina Human
- OmniExpressExome Array
- 1 million SNPs
- Somatic Chrom. Alterations

Targeted DNA Sequencing
- Illumina Trusight Tumor26
- 26 Cancer Associated Genes
- Deep Coverage (mean 6,000x)
- PE (2 x 150)

RNA Sequencing
- Illumina TruSeq Stranded
- Quantify Gene Expression
- PE (2 x 100)
Methods

DNA & RNA Extraction (Qiagen AllPrep DNA/RNA FFPE Kit)

Whole Genome SNP Genotyping
- Illumina Human OmniExpressExome Array
- 1 million SNPs
- Somatic Chrom. Alterations
Methods

Infinium Human OmniExpressExome Microarray
Results (Somatic Chromosomal Alterations)

B allele Frequency

\[
\text{B allele Frequency} = \frac{\text{Signal B Probe}}{\text{Signal A Probe} + \text{Signal B Probe}}
\]

- 1 (homozygote major allele) ‘CC’
- 0.5 (heterozygote) ‘CT’
- 0 (Homozygote minor allele ‘TT’)

Discovery Medicine 2018
Results (Somatic Chromosomal Alterations)

Example:
Participant 0609

Identification of UCS somatic chromosomal alterations (SCAs) that are shared, carcinoma-specific and sarcoma-specific

Light Blue – Carcinoma-specific SCAs
Dark Green – Sarcoma-specific SCAs
Results (Somatic Chromosomal Alterations)
First time separated UCS components subjected to molecular profiling utilizing a SNP microarray to measure SCA

A mean of 89% of the genomic alterations were shared between the carcinoma and sarcoma elements
  - 5.7% unique to carcinoma
  - 5.8% unique to sarcoma

Higher degrees of inter-component heterogeneity reflect more advanced disease

Implies a monoclonal origin of disease
Methods

DNA & RNA Extraction (Qiagen AllPrep DNA/RNA FFPE Kit)

Targeted DNA Sequencing
- Illumina Trusight Tumor26
- 26 Cancer Associated Genes
- Deep Coverage (mean 6,000x)
- PE (2 x 150)
DNA Sequencing

- **Whole Genome Sequencing**
  - 3 billion nucleotides (10 – 30 times)

- **Whole Exome Sequencing**
  - Protein coding region
  - ~ 50 million nucleotides (100x – 200x)

- **Targeted Region**
  - Defined by vendor (Trusight Tumor 26)
  - Can be defined by user
  - Size varies (20,000 – 550,000 nucleotides)
DNA Sequencing

1. Library Preparation
   - Input DNA
   - Fragmentation
   - Adaptor ligation

2. Bridge PCR

3. Sequencing by Synthesis
   - Template
   - Primer
   - Terminator caps
   - Fluorescent dye
   - Fluorescent emission
   - Cleavage
### Table 1: TruSight Tumor Genes

<table>
<thead>
<tr>
<th></th>
<th>AKT1</th>
<th>EGFR</th>
<th>GNAS</th>
<th>NRAS</th>
<th>STK11</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK</td>
<td>ERBB2</td>
<td>KIT</td>
<td></td>
<td></td>
<td>TP53</td>
</tr>
<tr>
<td>APC</td>
<td>FBXW7</td>
<td>KRAS</td>
<td></td>
<td></td>
<td>PIK3CA</td>
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<tr>
<td>BRAF</td>
<td>FGFR2</td>
<td>MAP2K1</td>
<td></td>
<td>PTEN</td>
<td></td>
</tr>
<tr>
<td>CDH1</td>
<td>FOXL2</td>
<td>MET</td>
<td></td>
<td></td>
<td>SMAD4</td>
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<tr>
<td>CTNNB1</td>
<td>GNAQ</td>
<td>MSH6</td>
<td></td>
<td></td>
<td>SRC</td>
</tr>
</tbody>
</table>

Genes selected from NCCN\(^1\) and CAP\(^2\) guidelines, late-stage clinical trials\(^3\), and relevant publications for lung, colon, melanoma, gastric and ovarian\(^4\).
Results (Targeted DNA Sequencing)
Summary (Targeted DNA Sequencing)

- Assess somatic variation
- All mutations previously described in COSMIC Database
- A total of 37 somatic mutations identified (18 tissues)
  - 1 – 3 mutations per samples
- TP53 most prevalent (Tumor protein p53)
  - 12/18 patients
  - Encodes a tumor suppressor protein
  - Protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism
  - Mutations in this gene are associated with a variety of human cancers
- PTEN (6/18), FBXW7 (4/18), FGFR2 (4/18), KRAS (4/18), PIK3CA (3/18), CTNNB1 (2/18)
Methods

DNA & RNA Extraction (Qiagen AllPrep DNA/RNA FFPE Kit)

RNA Sequencing
- Illumina TruSeq Stranded
- Quantify Gene Expression
- PE (2 x 100)
- Gene Expression
- 20,000+ genes analyzed
- Epithelial to Mesenchymal Transition (EMT)
- NGS Technology
EMT Analysis: 6 down-regulated genes and 5 up-regulated genes
2 pairs of samples discrepant in EMT status (0317 and 0622)
EMT important measure for assessing metastasis risk
Indicates clinical utility of separating the elements prior to analysis
Results (RNA Sequencing)
Gene Expression patterns between carcinoma and sarcoma samples revealed few differences.

Gene set analyses of differentially expressed genes failed to identify any component specific aberrant pathways.

**EMT**
- 9 samples EMT-like
- 7 samples epithelial-like
- More common in sarcoma (5/8 vs. 2/6)
- EMT-like status was associated with advanced stage (Fisher’s test, $p = 0.02$)
- EMT-like status was also associated with higher degrees of inter-component heterogeneity (Wilcoxin rank sum, $p = 0.03$)
Evolutionary Theories

Collision Theory
- Two Different Stem Cells
  - Sarcoma
  - Carcinoma
  - Uterine Carcinosarcoma

Combination Theory
- Common Stem Cell
  - Sarcoma
  - Carcinoma
  - Carcinoma
  - Uterine Carcinosarcoma

Conversion Theory
- Common Stem Cell
  - Carcinoma
  - Sarcoma
  - Uterine Carcinosarcoma
Summary

- UCS genomic profiling of distinct carcinoma and sarcoma components reveals deep similarities and implies monoclonal origin
- An EMT-like gene expression signature was observed in samples from advanced stage UCS patients
- Higher levels of genomic inter-component heterogeneity was associated with greater predicted metastatic potential
- Component-specific profiling may provide a means of refining clinical staging for UCS patients
Limitations and Future Plans

- **FFPE Tissue**
  - Degraded Nucleic Acids
- **Sample Size**
  - Rare Cancer
  - Homologous vs Heterologous
- Extend study to include Heterologous cases
- Incorporate methylation profiling (microarrays) to examine epigenetic influences on gene expression
- Add additional cases to increase power to detect differences in gene expression
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THANK YOU

FOR YOUR TIME.

QUESTIONS