Developing Liquid Biopsy for Predicting Early Disease & Therapeutic Drug Response in Localized & Metastatic Kidney Cancer

Viola Chen, MD
T32 update
Fan lab
1-23-19
Kidney cancer biomarker development

Localized disease (stage I/II)
- Platelet RNA-seq for detection of localized kidney cancer

Metastatic disease (stage IV)
- Nanotechnology capture of kidney cancer CTCs
Platelet RNA-seq for the early detection of kidney cancer
Achieving early kidney cancer detection

- Nanotechnology to molecularly probe for pre-malignant or early cancer lesions
- Next-generation sequencing to diagnose kidney cancers at stage I
Early diagnosis of kidney cancer means better survival

<table>
<thead>
<tr>
<th>AJCC Staging</th>
<th>5-year survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>96%</td>
</tr>
<tr>
<td>Stage II</td>
<td>82%</td>
</tr>
<tr>
<td>Stage III</td>
<td>64%</td>
</tr>
<tr>
<td>Stage IV</td>
<td>23%</td>
</tr>
</tbody>
</table>

Incidence (stage III&IV): 40%

Chittoria, Renal Cell Carcinoma, Cleveland Clinic CCE. Aug 2013
Platelets: beyond coagulation

- Megakaryocytes package RNA into platelets
  - 5000-9000 human genes

- Active translational machinery exists in platelets
  - process non-mature RNA into mature forms

- Bidirectional communication with environment
  - direct physical interactions
  - secretion/horizontal transfer microparticles
  - capture/sequestration circulating proteins/microvesicles
Platelets reflect a systemic response to disease

- Megakaryocytes pre-package unique repertoire of transcripts → platelets
- **Hypothesis:** early kidney cancer induces change in platelet RNA profiles
- Active splicing machinery allows processing pre-packaged RNA in response to environmental signals
- Disease-specific RNA patterns described in many diseases, including cancer
Finding a cancer-specific platelet RNA signature

- Gender-matched
- Race-matched
- Age-matched
Healthy donor platelet transcriptome stable over time
Platelet RNA-seq workflow

- Isolate platelets from whole blood of pilot patients
- Extract total RNA from platelets; convert to cDNA
- RNA-seq

Isolation of desired cell type

- Determine ideal digestion time for stranded RNA-seq of low-input material
- Determine candidate RNA signature for localized RCC
Sequencing low-input platelet RNA

Fragmentation based on RIN #
3 min digestion produces greatest % coding sequences

“MultiQC” Bioinformatics (2016)
Pre-pilot RNAseq shows excellent gene coverage
## Clinical sample collection status

### Paired Samples (Pre- & Post-Surgery)

<table>
<thead>
<tr>
<th>Clinical Diagnosis</th>
<th>Number of Patients</th>
<th>IDs</th>
<th>Number of Samples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear Cell RCC only</td>
<td>11</td>
<td>RCC230, RCC231, RCC234</td>
<td>22</td>
<td>2 time points each, stage I unless indicated otherwise</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTC713, CTC715, RCC250,</td>
<td></td>
<td>RCC234: stage II + stable lung nodules of unknown etiology, unlikely metastatic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RCC252, RCC254, RCC255, CTC726/RCC254, CTC725/RCC259</td>
<td></td>
<td>RCC255: stage II w/ rhabd. features + lung nodules, possibly metastatic, first seen on CT just before 2nd timepoint</td>
</tr>
<tr>
<td>Non-Clear Cell RCC only</td>
<td>3</td>
<td>CTC719, CTC724/RCC256, CTC722</td>
<td>6</td>
<td>CTC719: stage I pRCC type I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CTC724: stage II pRCC type II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CTC722: stage I clear cell tubulo-papillary RCC</td>
</tr>
<tr>
<td>RCC + Other Cancer</td>
<td>2</td>
<td>CTC711, CTC717/RCC240</td>
<td>5</td>
<td>CTC711: stage I ccRCC + bladder cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CTC717: 3 time points (3rd timepoint 5 months p surgery); stage I MIT translocation RCC + lung cancer</td>
</tr>
<tr>
<td>Benign Kidney Tumors</td>
<td>1</td>
<td>CTC716</td>
<td>1</td>
<td>CTC716: mixed epithelial and stromal tumor, likely benign</td>
</tr>
<tr>
<td>Non-Tumor Surgery</td>
<td>1</td>
<td>CTC1501</td>
<td>2</td>
<td>CTC1501: simple nephrectomy d/t hydrenephrosis</td>
</tr>
<tr>
<td>Healthy Controls</td>
<td>1</td>
<td>CTC590</td>
<td>2</td>
<td>(platelets)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>(plasma)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>20</td>
<td></td>
<td>41</td>
<td>(platelets)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td>(plasma)</td>
</tr>
</tbody>
</table>

### Single Time Points (pre-surgery/ non-surgery)

<table>
<thead>
<tr>
<th>Clinical Diagnosis</th>
<th>Number of Patients</th>
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<th>Number of Samples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear Cell RCC</td>
<td>1</td>
<td>RCC248</td>
<td>1</td>
<td>RCC248: stage I</td>
</tr>
<tr>
<td>Non-Clear Cell RCC</td>
<td>3</td>
<td>CTC209 (platelet RNA used up, only plasma left), CTC211, CTC2710, CTC721</td>
<td>4</td>
<td>CTC209: stage III chRCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CTC211: Xp11 translocation-associated renal cell carcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CTC710: stage I pRCC type II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CTC721: stage I chRCC</td>
</tr>
<tr>
<td>Benign Kidney Tumors</td>
<td>1</td>
<td>CTC716</td>
<td>1</td>
<td>CTC716: mixed epithelial and stromal tumor, likely benign</td>
</tr>
<tr>
<td>Non-Tumor Surgery Patients</td>
<td>1</td>
<td>CTC1500</td>
<td>1</td>
<td>CTC1500: simple nephrectomy d/t hydrenephrosis</td>
</tr>
<tr>
<td>Healthy Controls</td>
<td>3</td>
<td>CTC577, CTC576, CTC575</td>
<td>3</td>
<td>All male</td>
</tr>
<tr>
<td>TOTAL</td>
<td>9</td>
<td>(platelets)</td>
<td>10</td>
<td>(platelets)</td>
</tr>
</tbody>
</table>

- **Single Time Points (pre-surgery/ non-surgery)**
  - Clear Cell RCC
    - RCC248: stage I
  - Non-Clear Cell RCC
    - CTC209: stage III chRCC
    - CTC211: Xp11 translocation-associated renal cell carcinoma
    - CTC710: stage I pRCC type II
    - CTC721: stage I chRCC
  - Benign Kidney Tumors
    - CTC716: mixed epithelial and stromal tumor, likely benign
  - Non-Tumor Surgery Patients
    - CTC1500: simple nephrectomy d/t hydrenephrosis
  - Healthy Controls
    - All male
  - TOTAL
    - 9 (platelets)
    - 10 (plasma)
Conclusion

- Developed robust platelet isolation protocol from peripheral blood draw from clinic and pre-op patients
- Determined ideal digestion time for low-input total RNA stranded sequencing
- Completed collection of pilot paired-sample platelet patients with localized kidney cancer and additional control groups
- Poised to submit for sequencing and subsequent analysis
Acknowledgements

**Fan Lab**
Alice Fan, MD  
Viola Chen, MD  
Christian Hoerner, PhD

**Collaborators:**
Matthew T. Rondina, MD  
*University of Utah*

Emilie Montenont, PhD  
*University of Utah*

Jesse Rowley, PhD  
*University of Utah*

Neal Tolley  
*University of Utah*

University of Utah Bioinformatics Core  
*The Huntsman Cancer Institute*

Eric Oermann, MD  
*Mount Sinai Health System*

**Funding:**
- NCI T32: Dr. Rao, Dr. Felsher  
- TRAM grant  
- Canary Center for Early Cancer Detection

**TRAM**
Dean Felsher, MD  
Joanna Liliental, PhD

**Dept of Biomedical Data Science**
Laurel Stell, MS, PhD

**Dept of Urology**
Tommy Metzner, John Leppert, MD, Ben Chung, MD, Geoffrey Sonn, MD, Harcharan Gill, MD, Alan Thong, MD

**Stanford Cancer Center**
Chia-Sui Kao, MD  
Christian Kunder, MD  
Participating patients