Fluorescence in situ Hybridization (FISH) and Array-Comparative Genomic Hybridization (a-CGH) from Percutaneous Needle Biopsy Compared to Renal Mass Histology

Massimiliano Spalvieri1, Kelly L. Stratton1, Timothy F. Donahue1, Banumathy Gowrishankar*, Charles Ma*, Jeremy C. Durack1, Stephen B. Solomon1, Jane Houldsworth1, and Jonathan A. Coleman1
1Memorial Sloan-Kettering Cancer Center; *Cancer Genetics, Inc.

OBJECTIVE
To correlate the two novel assays Fluorescence in Situ Hybridization (FISH) and Array-Comparative Genomic Hybridization (a-CGH) with the histologic findings of kidney tumor biopsies.

INTRODUCTION
- Image-guided, percutaneous biopsy of kidney tumors is increasingly utilized, particularly in patients at higher risk of adverse outcomes;
- Biopsy results may facilitate decision-making in the management of small renal masses;
- Despite improved biopsy techniques, low yield and disrupted tissue architecture may make histologic diagnosis impossible;
- Specific genetic alterations have been identified in kidney tumors;1–5
- Accurate detection of genetic alterations may improve the diagnostic capabilities of percutaneous kidney biopsy;
- Selected patients may avoid extirpative treatment if benign or indolent tumors are determined by biopsy.

METHODS & RESULTS

FISH (FISHcore®) Analysis:
- Probe combinations used:
  - 15 probes per combination (Figure 3);
  - Minimum of 100 cells scored using epifluorescence microscope per hybridization;
  - 10 normal kidney specimens used to determine cut-off values;
- Histologic classification:
  - FISH decision tree (developed using an independent dataset of core biopsies)

FISH:
- Total of 23 biopsies
- 6 Biopsies used for optimization of fixation procedure
- 5 Biopsies classified as clear cell RCC (ccRCC)
- 4 Biopsies classified as papillary RCC (pRCC)
- 4 Non-diagnostic biopsies
- Lack of sufficient cells to generate score
- 2 Biopsies unclassifiable
- Alternates not consistent with the four cortical neoplasms included in the decision tree

RESULTS

Array-CGH:
- DNA extraction resulted in yields >500ng after QC
- Reference DNA: Sex-matched DNA (Promega)
- Digested and labeled DNA hybridized to targeted oligonucleotide microarray and analyzed according to manufacturer’s guidelines (Agilent Technologies)
- Identification of genomic aberrations:
  - Nexus Copy Number Analysis 6.1 (Bidi/Discovery Inc.)
  - Histologic classification:
    - a-CGH decision tree (developed using publicly available data)
- Copy number aberrations not related to four studied renal cortical neoplasms identified as Unclassifiable-RCC

Array-CGH Cases:
- Total of 23 biopsies
- 21 Biopsies analyzed
- 7 Biopsies classified as clear cell RCC (ccRCC)
- 8 Biopsies classified as papillary RCC (pRCC)
- 1 Biopsy classified as oncocytoma
- 3 Biopsies were unclassifiable
- 2 Biopsies: non-diagnostic

CONCLUSIONS
- Kidney biopsy can yield sufficient material for FISH and a-CGH studies.
- In this initial experience, the a-CGH method provided:
  - A more robust and interpretable assay than FISH;
  - Tumor classification with better correlation to pathologic assessment than FISH;
  - Less non-diagnostic results compared to FISH.
- Larger experience with these novel diagnostic tools is needed to determine their utility for the genomic classification of kidney tumors from kidney needle biopsies.

REFERENCES

Table 1. FISH and a-CGH vs. Biopsy or Nephronectomy Histology

<table>
<thead>
<tr>
<th>Biopsy Diagnosis</th>
<th>FISH Results</th>
<th>a-CGH Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Unclassifiable</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Oncocytoma</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Clear Cell RCC</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Papillary RCC</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Non-diagnostic</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Comparison of biopsy diagnosis by FISH and a-CGH with histology.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Biopsy Diagnosis</th>
<th>FISH Results</th>
<th>a-CGH Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>ccRCC</td>
<td>Concordant</td>
<td>Concordant</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Papillary RCC</td>
<td>Concordant</td>
<td>Concordant</td>
</tr>
<tr>
<td>Patient 3</td>
<td>Unclassifiable</td>
<td>Concordant</td>
<td>Concordant</td>
</tr>
<tr>
<td>Patient 4</td>
<td>Oncocytoma</td>
<td>Concordant</td>
<td>Concordant</td>
</tr>
</tbody>
</table>

CONFLICTS OF INTEREST
- B.G., C.M. and J.H. are full time employees of Cancer Genetics, Inc.

METRICS & RESULTS

Figure 1. FISH Assay Decision Tree

Figure 2. aCGH Based Decision Tree

Figure 3. Representative Images of FISH

Figure 4. Case Examples of Array-CGH Profiles from Core Biopsies.