Array-Comparative Genomic Hybridization (a-CGH)-Based Algorithm for Renal Tumor Subtyping in Needle Biopsies

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OBJECTIVE

- To develop a molecular assay to augment biopsy histology in subtyping renal cortical neoplasms.

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.

MATERIALS

Specimen acquisition:

- Percutaneous 18-22 Gauge core biopsies (n = 49) from 47 renal masses and 1 enlarged LN prospectively collected from 46 patients (11/2011 – 1/2014).
- Excluded cases:
  - Cystic fluid only (1 patient);
  - No extracted DNA (1 patient).
- Technique:
  - 1-4 core biopsies/tumor (median: 2);
  - 1-2 cores: DNA extraction for a-CGH.

Histologic Analysis:

- Diagnosis from pathology reports of biopsy tissue;
- Surgical pathology assessment used when available.

METHODS & RESULTS

Study Patient Characteristics:

- 27 Men, 19 Women
- Median Age (years): 72 (IQR: 63, 74)
- Median Tumor Size (cm): 2.7 (IQR: 1.9, 4.1)
- Median DNA extraction (μg): 2.28 (IQR: 0.89, 4.82)

Array-CGH:

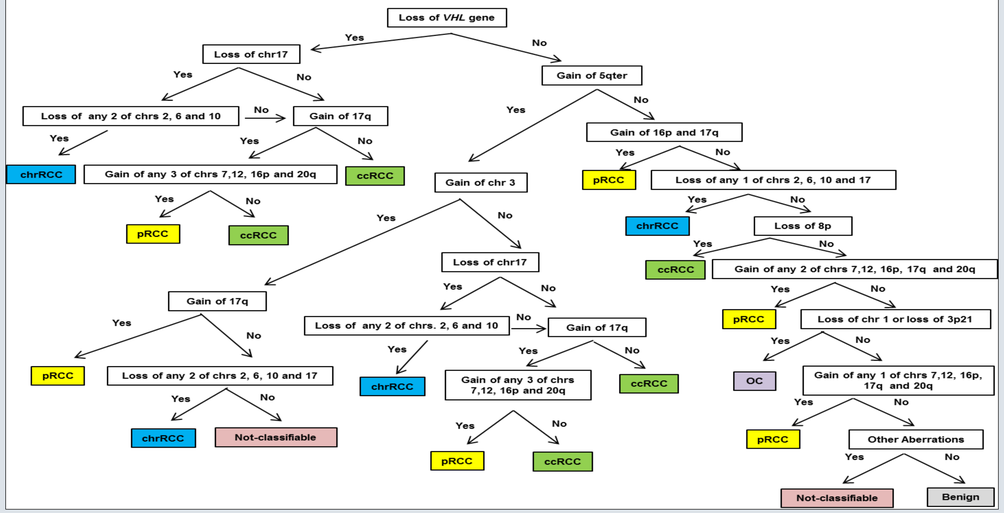
- DNA extraction resulted in yields >500 ng after QC (n = 41).
- Reference DNA: Sex-matched DNA (Promega).
- Digested and labeled DNA hybridized to targeted oligonucleotide microarray and analyzed according to manufacturer (Agilent Technologies).
- Identification of genomic aberrations:
  - Nexus Copy Number Analysis 7.5 (BioDiscovery 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 Not-Classifiable.
- Biopsies exhibiting no aberrations (other than normal variants) classified as Benign.

METHODS & RESULTS

Array-CGH Cases:

- Total of 47 biopsies from 44 patiens.
- Median maximum core size (cm): 0.7 (IQR: 0.5, 1.0)
- Pathologic Classification (n = 47):
  - Clear cell RCC (ccRCC) = 15
  - Papillary RCC (pRCC) = 11
  - Chromophobe RCC (chrRCC) = 2
  - Unclassified RCC = 3
  - Poorly differentiated favor RCC = 1
  - Low-grade oncocytic neoplasm = 4
  - Low-grade smooth muscle neoplasm = 1
  - Benign/Fibrosis = 3
  - Angiomyolipoma = 2
  - Oncocytoma = 2
  - High-grade urothelial carcinoma (UC) = 1
  - Non-diagnostic = 2
- Excluded cases = 2

aCGH-based Decision Tree



RESULTS

Comparison of biopsy diagnosis and aCGH with histology.

Sample	DNA (μg)	aCGH	Histology	Nephrectomy
Patient 1	0.21	ccRCC	ccRCC	NA
Patient 2	1.08	ccRCC	ccRCC	NA
Patient 3	0.91	ccRCC	ccRCC	ccRCC
Patient 4	2.30	ccRCC	ccRCC	ccRCC
Patient 5	0.53	ccRCC	ccRCC	ccRCC
Patient 6	2.04	ccRCC	ccRCC	ccRCC
Patient 7	2.62	ccRCC	ccRCC	ccRCC
Patient 8	10.51	ccRCC	Unclassified RCC	ccRCC
Patient 9	2.26	ccRCC	Unclassified RCC	ccRCC
Patient 10	7.75	ccRCC	Poorly Differentiated	NA
Patient 11 (Right Kidney)	5.55	Benign	ccRCC	NA
Patient 11 (Left Kidney)	2.21	pRCC	pRCC	NA
Patient 12	0.56	pRCC	pRCC	NA
Patient 13	4.92	pRCC	pRCC	Unclassified RCC
Patient 14	11.29	pRCC	pRCC	NA
Patient 15 (Initial)	4.34	Benign	Benign	NA
Patient 15 (Repeat)	11.57	pRCC	pRCC	NA
Patient 16	11.07	pRCC	pRCC	NA
Patient 17	9.73	pRCC	pRCC	NA
Patient 18	3.62	pRCC	pRCC	NA
Patient 19	5.41	pRCC	pRCC	pRCC
Patient 20	21.45	pRCC	pRCC	NA
Patient 21	0.48	pRCC	Unclassified RCC	NA
Patient 22	4.21	pRCC	Low Grade Oncocytic Neoplasm	NA
Patient 23	3.80	pRCC	Low Grade Smooth Muscle Neoplasm	NA
Patient 24	0.86	pRCC	Benign	NA
Patient 25	4.10	chrRCC	chrRCC	chrRCC
Patient 26	4.50	chrRCC	chrRCC	NA
Patient 27	2.16	chrRCC	Oncocytoma	NA
Patient 28 (Kidney)	2.30	Benign	ccRCC	NA
Patient 28 (Lymph Node)	13.67	Not Classifiable	ccRCC	NA
Patient 29	1.56	Not Classifiable	ccRCC	ccRCC
Patient 30	0.46	Benign	ccRCC	NA
Patient 31	0.47	Not Classifiable	Not Diagnostic	NA
Patient 32	5.22	Benign	Not Diagnostic	NA
Patient 33	0.57	Benign	ccRCC	NA
Patient 34	1.56	Benign	pRCC	NA
Patient 35	4.55	Benign	Low Grade Oncocytic Neoplasm	NA
Patient 36	0.93	Benign	Low Grade Oncocytic Neoplasm	NA
Patient 37	1.77	Benign	Low Grade Oncocytic Neoplasm	NA
Patient 38	2.89	Benign	Fibrosis	NA
Patient 39	1.65	Benign	Angiomyolipoma	NA
Patient 40	1.57	Benign	Angiomyolipoma	NA
Patient 41	0.47	Not Diagnostic	ccRCC	NA
Patient 42	0.06	Not Diagnostic	ccRCC	ccRCC
Patient 43	0.02	Not Diagnostic	Oncocytoma	NA
Patient 44	0.05	Not Diagnostic	Urothelial Carcinoma	NA

Legend

Concordant	Not Concordant	Discordant	Not Applicable

CONCLUSIONS

- DNA yields ≤0.06 μg impaired often aCGH diagnostic capabilities.
- Overall concordance between aCGH and histology of kidney biopsy or surgical specimen was 60%.
- However, the concordance between the aCGH subtyping and surgical specimen histology was 90%.
- Other interesting observations:
  - aCGH was able to offer a definitive diagnosis (confirmed by histologic examination of the surgical specimen) for 2 specimens (patients #8 and #9) that were called unclassified RCC by biopsy histology.
  - Considering the overlapping morphologic features between chrRCC and OC and the difficult discrimination between these two entities based on histology alone, histology called a specimen (patient #27) as OC while molecular classification by aCGH for the same specimen was chrRCC.
  - The clinical behavior of oncocytomas, which usually present quiet genomic changes, and low-grade oncocytic neoplasms, which are poorly understood, is benign. However, low-grade oncocytic neoplasms have the potential to be mixed with smaller components of more aggressive neoplasms. aCGH identified aberrations related to a malignant subtype in one of the four low-grade oncocytic neoplasms in this study.
- Genomic-based platforms have the potential to play a significant role in augmenting histopathology findings from core biopsy.

CONFLICTS OF INTEREST

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

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