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

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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.

MATERIALS

Specimen acquisition:

➢Percutaneous 18-22 Gauge core biopsies from 25 renal masses prospectively collected from 22 patients (9/2011 – 1/2013)

➢Excluded cases:
- ➢ Clinical data (1 patient); Cystic fluid only (1 patient).

➢ Technique:
- ➢ 1-4 core biopsies/tumor (median: 2)
- ➢ 1 core fixed for FISH
- ➢ 1-2 cores: DNA extraction for a-CGH

Study Patient Characteristics:

➢ 12 Men, 8 Women
➢ Median Age: 70 (IQR: 63, 74)
➢ Median Tumor Size: 3.1 cm (IQR: 2.0, 5.9)

Histologic Analysis:

➢ Diagnosis from pathology reports of biopsy tissue
➢ Surgical pathology assessment used when available

METHODS & RESULTS

FISH (FReCaD™) Analysis:

➢ 7-Probe combinations used
- ➢ Up to 3 colors 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)

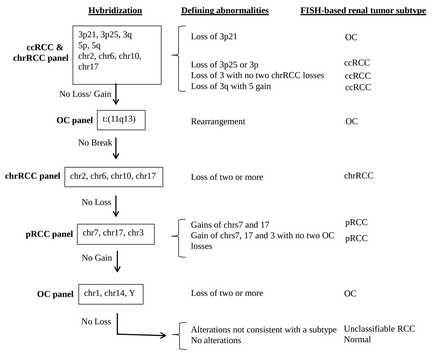
FISH:

➢ 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)
➢ 6 Non-diagnostic biopsies
- ➢ Lack of sufficient cells to generate score

➢ 2 Biopsies unclassifiable
- ➢ Aberrations not consistent with the four cortical neoplasms included in the decision tree

Figure 1.

FISH Assay Decision Tree



METHODS & 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 (Agilent Technologies)
➢ Identification of genomic aberrations:
- ➢ Nexus Copy Number Analysis 6.1 (BioDiscovery Inc.)

➢ Histologic classification:
- ➢ a-CGH decision tree (developed using publicly available data)

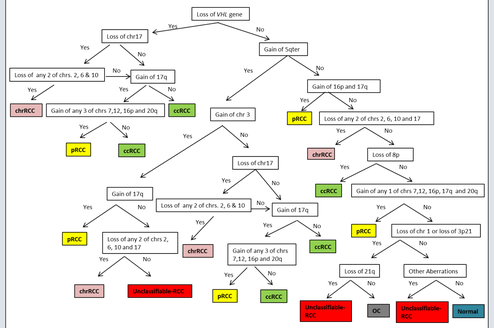
➢ Copy number aberrations not related to four studied renal cortical neoplasms identified as Unclassifiable-RCC
➢Biopsies exhibiting no aberrations (>2Mb) classified as normal

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

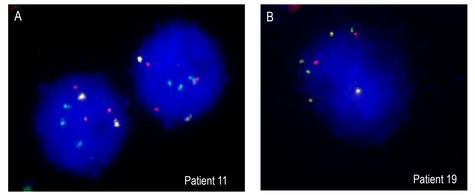
Figure 2.

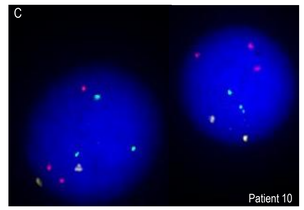
aCGH-Based Decision Tree



RESULTS

Figure 3: Representative Images of FISH



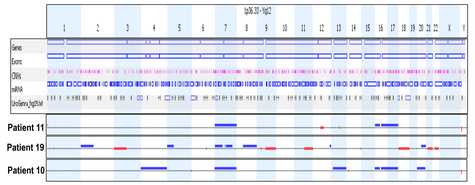


A) Patient 11: gain of chrs 7 and 17 (pRCC).

B) Patient 19: loss of 3p with respect to control probe on the same chromosome (ccRCC).

C) Patient 10: gain of chrs 7 and 17 (pRCC).

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



Blue = Gain
Red = Loss

Patients10 and 11: Classified as Papillary RCC; Patient 19: Classified as Clear Cell RCC

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

Assay	Histology	
	Concordant	Discordant
FISH	5	5
A-CGH	15	3

➢ Non-diagnostic results affected both FISH and a-CGH

RESULTS

Table 2. Comparison of biopsy diagnosis by FISH and aCGH with histology.

Sample	FISH	aCGH	Histology	Nephrectomy
Patient 1	Optimization	Oncocytoma	Oncocytoma	NA
Patient 2	Non-Diagnostic	Normal	Angiomyolipoma	NA
Patient 3	Non-Diagnostic	Non-Diagnostic	Oncocytoma	NA
Patient 4	ccRCC	Non-Diagnostic	Urothelial Carcinoma	NA
Patient 5	Unclassifiable RCC	pRCC	Unclassified RCC	NA
Patient 6	pRCC	pRCC	NA	NA
Patient 7 (Initial)	Non-Diagnostic	Normal	Benign	NA
Patient 7 (Repeat)	Unclassifiable RCC	pRCC	pRCC	NA
Patient 8	ccRCC	pRCC	pRCC	NA
Patient 9	ccRCC	pRCC	pRCC	Unclassified RCC
Patient 10	pRCC	pRCC	pRCC	NA
Patient 11	pRCC	pRCC	pRCC	NA
Patient 12	pRCC	pRCC	pRCC	NA
Patient 13	Optimization	ccRCC	NA	NA
Patient 13	Optimization	ccRCC	ccRCC	NA
Patient 14	Optimization	Unclassifiable RCC	ccRCC	NA
Patient 14(Lymph node)	Optimization	Unclassifiable RCC	ccRCC	NA
Patient 15	Optimization	ccRCC	ccRCC	NA
Patient 16	Non-Diagnostic	ccRCC	Unclassified RCC	ccRCC
Patient 17	Non-Diagnostic	Unclassifiable RCC	ccRCC	ccRCC
Patient 18	Non-Diagnostic	ccRCC	ccRCC	ccRCC
Patient 19	ccRCC	ccRCC	ccRCC	ccRCC
Patient 20	ccRCC	ccRCC	ccRCC	ccRCC

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 interpretive 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.

CONFLICTS OF INTEREST

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

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