

Clinical Utility of a Custom Next-Generation Sequencing Panel in the Diagnosis of Needle Biopsies from Renal Masses

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INTRODUCTION

Image-guided, percutaneous biopsy of kidney tumors is increasingly utilized, particularly in patients at higher risk of adverse outcomes.

Despite improved biopsy techniques, low yield and disrupted tissue architecture may make histologic diagnosis challenging.

Selected patients may avoid extirpative treatment if benign or indolent tumors are determined accurately by biopsy.

Specific mutations and copy number changes have been identified in kidney tumors which could be utilized in subtyping and outcome prediction¹⁻⁴.

Simultaneous evaluation of mutation and copy number could be highly useful in providing both diagnostic and prognostic information from minimal biopsy material.

MATERIALS & METHODS

Patient Characteristics

48 percutaneous 18-22 gauge core needle biopsies prospectively collected from 45 patients (11/2011 – 1/2014)

28 Men, 20 Women

Median age (years): 72 (IQR: 63, 74)

Median Mass Size (cm): 2.7 (IQR: 1.0, 10.4)

DNA Yield: 20 ng – 21 ug

Molecular Analyses

aCGH (200 ng – 1 ug gDNA)
n=48 (43 diagnostic)

Blinded copy number-based molecular classification⁶

NGS with leftover DNA (25 ng – 250 ng)
n=41 (41 diagnostic)

Copy number-based molecular classification⁶

MATERIALS & METHODS

Targeted aCGH (UroGenRA[®])

- Custom array on Agilent 4x44K platform⁶
- Copy number changes identified by Rank in Nexus Copy Number 7.5 (BioDiscovery)
- Subtyped based on 15 genomic gains/losses⁶

Targeted Renal NGS Panel (Focus::Renal[™])

76 Genes Mutated in ccRCC

ABL1	BRAF	DIO2	GOLGA5	MAGEC1	PDGFRB	RASSF2	SOX4	UBE2D1
AFF1	BRINP2	DST	GUSB	MAPK14	PGLYRP3	RBAK	SPRED1	VHL
AKT1	CARD11	EGFR	HIF1A	MET	PIK3CA	RHEB	SRC	ZFPM2
AKT2	CCDC120	EPHB4	HSP90AA1	MTOR	PIK3CG	ROS1	STAG2	ZNF800
ALK	CCND2	ERBB2	IFNB1	NDRG1	PTCH1	SCARB2	TET2	
ARID1A	CDH4	FGFR1	ING4	NPNT	PTEN	SETD2	TNC	
AXL	CUL1	FLT3	JAK2	PBRM1	RAF1	SFXN4	TP53	
BAP1	DAXX	FYN	KDM5C	PCK1	RALGAPA1	SMO	TSC1	
BMPR1B	DFNA5	GLI3	KIT	PCNA	RAPGEF1	SMOX	TSC2	

16 RCC Prognostic SNPs

rs3834129 (CASP8)	rs4073 (CXCL8)	rs9582036 (VEGFR1)	rs7121 (GNAS)	rs1332018 (GSTM3)
rs11549465 (HIF1A)	rs2057482 (HIF1A)	rs1126647 (IL8)	rs11762213 (MET)	rs3814055 (NR1H2)
rs6785049 (NR1H2)	rs1054190 (SXR)	rs699947 (VEGF)	rs833061 (VEGF)	rs1570360 (VEGF)
rs3025039 (VEGF)				

PLUS: 3 Mb Backbone SNP probes

- Hybrid capture library enrichment (Nimblegen)
- Sequencing with MiSeq (Illumina)
- Variant identification – CLCbio (Qiagen)
- Gain/loss estimation – CNVKit⁵
- Subtyped based on 15 genomic gains/losses⁶

Alignment and Somatic Variant Calling
 • CLC Biomedical Genomics Workbench
 • Human ref genome hg19/GRCh37

Initial Filtering
 • dbSNP, 1000 Genomes, HapMap MAF >0.1%

Final Filtering
 • Non-synonymous and noncoding
 • Homopolymer regions
 • SIFT and PolyPhen prediction (T and B)

ABBREVIATIONS

aCGH-array comparative genomic hybridization; AML-angiomyolipoma; ccRCC-clear cell renal cell carcinoma; chrRCC-chromophobe RCC; Cryo-cryoablation; HG-high grade; LG-low grade; NonD-non-diagnostic; NGS-next generation sequencing; Obs-observation; OC-oncocytoma; ON-oncocytic neoplasm; pRCC-papillary RCC; RCC-renal cell carcinoma; SMN-smooth muscle neoplasm; UC-urothelial carcinoma; UnRCC-unclassified RCC.

RESULTS

Histology and Copy Number-Based Subtyping

ccRCC (n=16):

#	Needle Biopsy Histology	Follow-up	Copy Number-Based Subtype	
			aCGH	NGS
11-B	ccRCC	Cryo	Benign	Benign
33	ccRCC	Cryo	Benign	Benign
29	ccRCC	ccRCC	Benign	Benign
28-A	ccRCC	Embolization	Benign	ccRCC
30	ccRCC	Obs	Benign	ccRCC
4	ccRCC	ccRCC	ccRCC	ccRCC
6	ccRCC	ccRCC	ccRCC	ccRCC
7	ccRCC	ccRCC	ccRCC	ccRCC
3	ccRCC	ccRCC	ccRCC	ccRCC
5	ccRCC	ccRCC	ccRCC	ccRCC
1	ccRCC	Obs	ccRCC	Not Benign
2	ccRCC	Everolimus	ccRCC	
42	ccRCC	ccRCC	NonD	
41	ccRCC	Cryo	NonD	
8	UnRCC	ccRCC	ccRCC	ccRCC
9	UnRCC	ccRCC	ccRCC	pRCC

Other Malignant RCC (n=17):

#	Needle Biopsy Histology	Follow-up	Copy Number-Based Subtype	
			aCGH	NGS
34	pRCC	Cryo	Benign	Benign
11-A	pRCC	Cryo	pRCC	
20	pRCC	Cryo	pRCC	pRCC
16	pRCC	Partial Nx	pRCC	pRCC
18	pRCC	Cryo	pRCC	pRCC
14	pRCC	Cryo	pRCC	pRCC
15-B	pRCC	Cryo	pRCC	pRCC
12	pRCC	Obs	pRCC	pRCC
17	pRCC (type 1)	Obs	pRCC	pRCC
19	pRCC (type 1)	pRCC	pRCC	pRCC
13	pRCC	UnRCC	pRCC	pRCC
25	chrRCC	chrRCC	chrRCC	chrRCC
26	chrRCC	Cryo	chrRCC	chrRCC
28-B	HG RCC	Sunitinib	Not Benign	Benign
10	Poorly diff. RCC	Temsirolimus	ccRCC	ccRCC
44	HG UC	NUx	NonD	
21	UnRCC	Temsirolimus	pRCC	pRCC

Benign Neoplasms/Non-Diagnostic (n=15):

#	Needle Biopsy Histology	Follow-up	Copy Number-Based Subtype	
			aCGH	NGS
43	OC	Obs	NonD	
27	OC	Obs	chrRCC	chrRCC
35	LG ON and AML	Obs	Benign	Benign
22	LG ON	Obs	pRCC	pRCC
37	LG ON favor OC	Obs	Benign	Benign
36	LG ON favor OC	Obs	Benign	OC
23	LG SMN favor AML	Obs	pRCC	pRCC
39	AML	Obs	Benign	Benign
40	AML	Obs	Benign	Benign
45	Benign	Obs	NonD	Benign
24	Benign	Obs	Benign	pRCC
15-A	Benign	Cryo	Benign	Benign
38	Fibrosis	Obs	Benign	Benign
31	NonD	Obs	Benign	
32	NonD	Obs	Benign	Benign

CONFLICTS OF INTEREST

B.G., M.J., V.T., and J.H. are full time employees and stock/stock option holders of Cancer Genetics, Inc. R.S.K.C is a Board member, paid consultant and stock/stock option holder for Cancer Genetics, Inc.

RESULTS

Concordance Between Histology and Copy Number-Based Subtyping

Total number needle biopsies – 48
 No. of diagnostic biopsies by NGS/aCGH – 41

For molecular subtyping, NGS copy number classification used when available, otherwise, aCGH classification was used for concordance estimates

Specimen Histology (Resected specimen histology used when performed; otherwise needle biopsy histology used)	Copy Number-based Subtype (n=41)	
	n	Concordant
ccRCC	14	9
Other Malignant RCC	15	12
pRCC	9	8
chrRCC	2	2
UnRCC	2	-
Poorly diff. /HG RCC	2	-
Benign Neoplasms	12	8
OC	1	-
LG ON	5	3
AML	2	2
Benign/Fibrosis	4	3

Reasons for mis-classification:

- low tumor burden
analytical sensitivity for aCGH/NGS copy number is ~20%
- rarely studied subtypes
many of the subtypes in this cohort are rare neoplasms and their copy number changes are not well documented
- genomic complexity
several biopsies in this cohort have mixed histologies and may have complex genomic imbalance

Evaluation of mutational changes in needle biopsies may throw additional insight into the malignant nature of the specimen

REFERENCES

- Klatte T, et al.: Cytogenetic profile predicts prognosis of patients with clear cell renal cell carcinoma. J Clin Oncol 2009, 27: 746-753.
- Matsuda D, et al.: Identification of copy number alterations and its association with pathological features in clear cell and papillary RCC. Cancer Lett 2008, 272: 260-267.
- Klatte T, et al.: Cytogenetic and molecular tumor profiling for type 1 and type 2 papillary renal cell carcinoma. Clin Cancer Res, 2009, 15: 1162-1169.
- Network, T. C. G. A.: Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature, 499: 43, 2013.
- Talevich E, et al.: CNVkit: Copy number detection and visualization for targeted sequencing using off-target reads. (Manuscript in preparation, 2014).
- Gowrishankar B, et al.: A genomic algorithm for the molecular classification of common renal cortical neoplasms: Development and Validation. J Urol. 2015, 193(5):1479-85.

RESULTS

Mutational Analysis of Needle Biopsies (n=41)

ccRCC (n=13):

#	Copy Number-Based Subtype	11-B	33	29	28-A	30	4	6	7	3	5	1	8	9
		Benign	Benign	Benign	ccRCC	ccRCC	ccRCC	ccRCC	ccRCC	ccRCC	ccRCC	ccRCC	ccRCC	ccRCC
Significantly mutated in RCC	VHL													
	PBRM1													
	BAP1													
	SETD2													
	TP53													
	KDM5C													
Other	ARID1A													
	HIF1A													
	MET													
	TSC1													
	TSC2													
	MTOR													

Other Malignant RCC (n=15):

#	Copy Number-Based Subtype	34	20	16	18	14	15-B	12	17	19	25	26	28-B	10	13	21
		Benign	pRCC	pRCC	pRCC	pRCC	pRCC	pRCC	pRCC	chrRCC	chrRCC	Benign	ccRCC	pRCC	pRCC	
Significantly mutated in RCC	VHL															
	PBRM1															
	BAP1															
	SETD2															
	TP53															
	KDM5C															
Other	ARID1A															
	HIF1A															
	MET															
	TSC1															
	TSC2															
	MTOR															

Benign/Non-Diagnostic (n=13):

#	Copy Number-Based Subtype	27	35	22	37	36	23	39	40	45	24	15-A	38	32
		chrRCC	Benign	pRCC	Benign	OC	pRCC	Benign	Benign	Benign	pRCC	Benign	Benign	Benign
Significantly mutated in RCC	VHL													
	PBRM1													
	BAP1													
	SETD2													
	TP53													
	KDM5C													
Other	ARID1A													
	HIF1A													
	MET													
	TSC1													
	TSC2													
	MTOR													

Mutational Analysis:

- detected malignant-related mutations in several samples deemed benign by copy number-based subtyping
- identified mutations of prognostic (BAP1) and therapeutic significance (MET and MTOR)