Metastasis-Associated Mutations in Clear Cell Renal Cell Carcinoma

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INTRODUCTION

About 70% of ccRCC patients are diagnosed as Stage I-III; 20-30% of these relapse within 3 years post nephrectomy[1].

Estimated 5-year survival rates are above 60% for Stage I-III and 23% for Stage IV[2].

While Stages I-III are treated by nephrectomy, metastatic patients require additional therapy[3].

Choice of therapy depends on the prognosis and site of metastatic spread.

ccRCC is characterized by several somatic mutations of prognostic and therapeutic significance[4, 5]. But mutations pertaining to metastasis/relapse and site of metastasis are not clearly understood.

Identification of novel somatic variants would be beneficial in selecting metastatic patients for appropriate therapy.

MATERIALS and METHODS

- Surgically resected primary and metastatic ccRCC (unmatched, fresh frozen) specimens (n=128) were acquired from Memorial Sloan-Kettering Cancer Center in this IRB-approved study.
- Specimen characteristics of the cohort are provided below:

Primary (n=78)

Clinical Stage	No. of Specimens
Stage I-III	29
Stage IV	30
Unknown	19

Metastatic (n=50)

Site of Metastasis	No. of Specimens
Lung	10
Bone	9
Other	25
Unknown	6

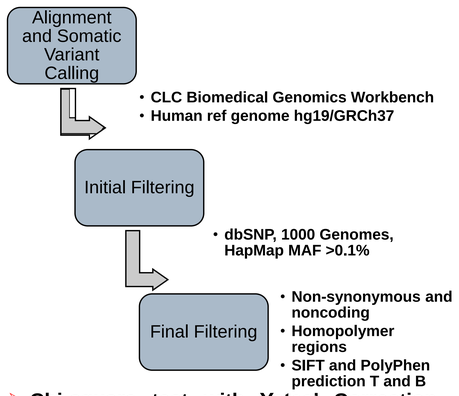
- Nimblegen hybrid-capture design encompassing 2400 targets (exonic regions of 76 genes, 16 prognostic SNPs, and backbone probes placed at 3 Mb apart for copy number estimation).

MATERIALS and METHODS

Focus::Renal™ Targeted NGS Panel:

Gene	Gene	Gene	Gene	SNP ID
VHL	IFNB1	HSP90AA1	PIK3CG	rs3834129
PBRM1	AKT1	PCK1	ZNF800	rs9582036
SETD2	SRC	CDH4	SMO	rs1332018
BAP1	CARD11	SCARB2	UBE2D1	rs7121
ARID1A	DFNA5	AFF1	PTEN	rs11549465
TP53	GLI3	BMPR1B	SFXN4	rs2057482
MTOR	GUSB	TET2	AKT2	rs3814055
PIK3CA	MET	NPNT	AXL	rs6785049
TSC1	CUL1	CCDC120	CCND2	rs1054190
TSC2	RHEB	KDM5C	ING4	rs699947
RBAK	ZFPM2	STAG2	HIF1A	rs833061
FGFR1	NDRG1	MAGEC1	ALK	rs1570360
PGLYRP3	PTCH1	SOX4	BRAF	rs3025039
BRINP2	TNC	DAXX	RAF1	rs1126647
SPRED1	ABL1	MAPK14	EGFR	rs4073
SMOX	RAPGEF1	DST	ERBB2	rs11762213
RASSF2	RALGAPA1	FYN	PDGFRB	
PCNA	DIO2	ROS1	KIT	
JAK2	GOLGA5	EPHB4	FLT3	

- Libraries prepared using Kapa HyperPrep kit
- Sequencing performed on an Illumina sequencing platform (Miseq)
- Variants identified using CLCbio (Qiagen)



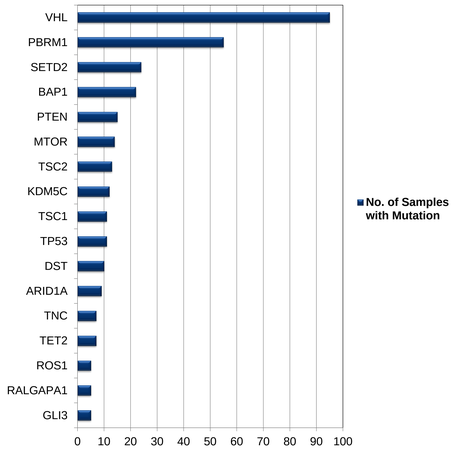
- Chi-square test with Yates' Correction was applied to test for significance

CONFLICTS OF INTEREST

B.G., M.J., V.T., C.M., A.G. 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 of Cancer Genetics, Inc.

RESULTS

Frequently Mutated Genes in 128 ccRCC



Primary vs Metastatic Mutations:

	Primary (n=78)		Metastatic (n=50)	
Gene	Mutation	None	Mutation	None
ARID1A	5	73	4	46
BAP1	13	65	9	41
DST	5	73	5	45
GLI3	2	76	3	47
KDM5C	6	72	6	44
MTOR	6	72	8	42
PBRM1	36	42	19	31
PTEN	10	68	5	45
RALGAPA1	3	75	2	48
ROS1	3	75	2	48
SETD2	13	65	11	39
TET2	3	75	4	46
TNC	5	73	2	48
TP53	6	72	5	45
TSC1*	3	75	8	42
TSC2	10	68	3	47
VHL	56	22	39	11

* Statistically significant

- TSC1 mutation significantly enriched in metastatic lesions (p=0.04)
- TSC1 maps to 9q34; Loss of 9q was significantly enriched in metastatic lesions by aCGH (reported at GU-ASCO 2015)
- 5/11 with TSC1 mutation also showed 9q loss

RESULTS

Stage I-III Primary vs Metastatic Mutations:

	Stage I-III Primary (n=29)		Metastatic (n=50)	
Gene	Mutation	None	Mutation	None
ARID1A	0	29	4	46
BAP1	4	25	9	41
DST	1	28	5	45
GLI3	2	27	3	47
KDM5C	2	27	6	44
MTOR	2	27	8	42
PBRM1	14	15	19	31
PTEN	4	25	5	45
RALGAPA1	1	28	2	48
ROS1	1	28	2	48
SETD2	4	25	11	39
TET2	0	29	4	46
TNC	2	27	2	48
TP53	3	26	5	45
TSC1*	0	29	8	42
TSC2	4	25	3	47
VHL	23	6	39	11

* p-value=0.06

Site-Enriched Mutations:

	Lung (n=10)		Other (n=34)	
Gene	Mutation	None	Mutation	None
ARID1A	0	10	2	32
BAP1	2	8	6	28
DST	2	8	1	33
GLI3	0	10	3	31
KDM5C	3	7	3	31
MTOR	2	8	5	29
PBRM1	5	5	12	22
PTEN	3	7	2	32
RALGAPA1	0	10	1	33
ROS1	1	9	1	33
SETD2	0	10	10	24
TET2	1	9	3	31
TNC	0	10	2	32
TP53	2	8	3	31
TSC1	0	10	8	26
TSC2	0	10	3	31
VHL	10	0	25	9

- None of the lung-enriched mutations were statistically significant

RESULTS

	Bone (n=9)		Other (n=35)	
Gene	Mutation	None	Mutation	None
ARID1A*	2	7	0	35
BAP1	1	8	7	28
DST	0	9	3	32
GLI3	1	8	2	33
KDM5C	0	9	6	29
MTOR	2	7	5	30
PBRM1	3	6	14	21
PTEN	0	9	5	30
RALGAPA1	1	8	0	35
ROS1	0	9	2	33
SETD2	3	6	7	28
TET2	0	9	4	31
TNC	1	8	1	34
TP53	0	9	5	30
TSC1	0	9	8	27
TSC2	1	8	2	33
VHL	6	3	29	6

* Statistically significant

- ARID1A mutation significantly enriched in metastatic bone lesions (p=0.05).

CONCLUSIONS

- A set of 17 genes were found to be mutated in primary and metastatic ccRCC lesions.
- TSCI mutation is significantly enriched in metastatic lesions consistent with our copy number findings where 9q loss was significantly found in metastatic lesions.
- Across metastatic lesions, ARID1A mutations were significantly enriched in ccRCC metastasis to bone.
- Genomic signatures identified in this study has the potential to identify patients with high risk of metastasis.

REFERENCES

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