Identification of Genomic Alterations Associated with Metastasis in Clear Cell Renal Cell Carcinoma (ccRCC)

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CANCER GENETICS
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INTRODUCTION & OBJECTIVE

- Clear cell renal cell carcinoma (ccRCC) is the most abundant subtype accounting for 70% of all renal cortical neoplasms
- Metastatic ccRCC is well known for its aggressive nature and poor prognosis with about 20-40% relapsing within 5 years of nephrectomy[1]
- Also, metastatic ccRCC is largely refractory to conventional treatments with a five-year survival rate 8-9%[2]. Targeted therapies have proven to increase the overall survival rate and treatment options vary depending on the site of metastasis[3]
- ccRCC is characterized by a series of genetic alterations that could be utilized for improving diagnosis and prognosis[4,5]
- The aim of the study is to identify genomic copy number alterations by high resolution approach (whole genome array-CGH) that could serve as biomarkers for metastasis

MATERIALS

Specimens:

- Fresh frozen surgically resected ccRCC (primary and metastatic) specimens were acquired from Memorial Sloan-Kettering Cancer Center (MSKCC) upon IRB approval
- Sample characteristics of the cohort are provided below:

Characteristics	Primary (n=115)	Metastatic (n=63)
Unmatched	81	29
Matched Pairs	34	34
Site of Metastasis (n>10)		
Lung	N/A	15
Brain	N/A	11
Other	N/A	29
Unknown	N/A	8

- aCGH profile data from 437 ccRCC specimens from TCGA as validation cohort. It comprises of 368 stage I, II, III and 69 stage IV specimens

METHODS

Whole Genome Array-CGH:

- DNA extraction resulted in yields >500ng after QC
- Reference DNA: Sex-matched DNA (Promega)
- Digested and labeled DNA hybridized to whole genome oligonucleotide microarray (244K) and analyzed according to the manufacturer (Agilent Technologies)

Analysis:

- Identification of genomic aberrations:
  - Used +-0.13 log ratio threshold to define gain and loss for the segments defined by Rank segmentation algorithm using Nexus Copy Number 7.5 (Biodiscovery Inc.)
  - Further analysis to identify significant differential aberrations between Primary and Metastatic groups using Fisher exact test with P-value <=0.05 (FDR)
  - Joined the segments if the segments were broken with several gaps with few MB apart with the neighboring segments.
  - Significant aberrations were identified based on the above criteria and with at least 15% differential frequencies between the groups

Approaches:

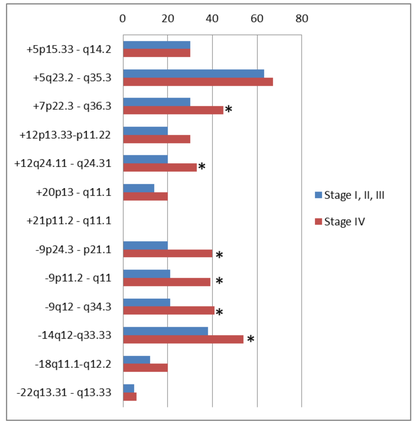
- Approaches to Identify Metastatic Markers:
  - Unmatched:
    81 Primary Vs 63 Metastatic
  - Matched Pairs:
    34 Primary Vs 34 Metastatic
- Validation of Metastatic Markers:
  - Stage I, II & III Primary ccRCC (TCGA Dataset) (n=368)
  - Stage IV Primary ccRCC (TCGA Dataset) (n=69)
  - Differential significance evaluated using Fisher exact test with P-value <=0.05
- Analysis of Site-specific Markers:
  - 15 Lung Metastatic Vs 40 Other sites
  - 11 Bone Metastatic Vs 44 Other sites

Unmatched Meta (63) *vs* Pri (81) Analysis

Table 1:
Aberrations occurring significantly higher in unmatched metastatic vs primary samples.

Cytoband	Chr	Start	End	Meta (%)	Pri (%)	Diff. Freq.
Gain						
5q22.1 - q31.2	5	111,088,102	137,658,174	54	35	19
5q33.3 - q35.3	5	156,200,073	176,849,793	61	43	18
7p11.2-7q11.2	7	55,773,108	65,211,013	30	13	17
20q11.1 - q13.12	20	27,500,000	45,248,483	32	13	19
21p11.2 - q11.1	21	10,874,759	13,200,000	19	2	16
Loss						
4p15.2 - p14	4	26,237,906	37,924,365	26	10	16
4q13.2 -35.2	4	75,519,857	176,135,971	27	12	15
9p24.3 - q11	9	0	49,000,000	39	21	18
9q21.12 - q21.33	9	73,749,752	90,080,594	39	23	16
14q32.33	14	96,794,393	107,349,540	45	26	19
18p11.31 - p11.22	18	4,711,281	10,830,535	25	10	15
18q11.2 - q23	18	17,200,000	78,077,248	29	12	17

Figure 1:
Frequency of significant aberrations in primary TCGA samples: State I, II, III versus Stage IV



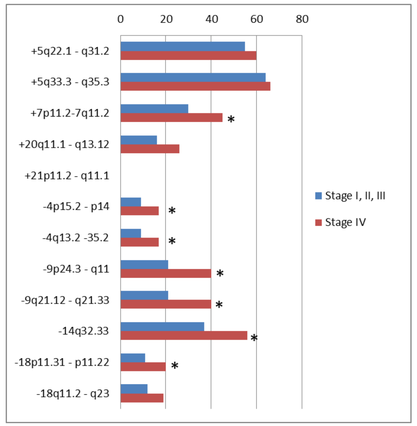
* Aberrations identified in unmatched dataset occurring significantly (**p-value <=0.05**; Fisher Exact test) higher in TCGA stage IV vs stage I, II, III

Matched Meta (34) *vs* Pri (34) Analysis

Table 2:
Aberrations occurring significantly higher in matched metastatic vs primary samples.

Cytoband	Chr	Start	End	Region Length	Diff. Freq.
Gain					
5p15.33 - q14.2	5	0	82,522,244	82,522,244	18
5q23.2 - q35.3	5	125,659,649	180,915,260	55,255,611	18
7p22.3 - q36.3	7	0	159,138,663	159,138,663	21
12p13.33-p11.22	12	0	28,608,557	28,608,557	18
12q24.11 - q24.31	12	110,276,239	125,291,346	15,015,107	18
20p13 - q11.1	20	0	27,500,000	27,500,000	18
21p11.2 - q11.1	21	10,874,759	13,200,000	2,325,241	21
Loss					
9p24.3 - p21.1	9	0	33,127,424	33,127,424	18
9p11.2 - q11	9	43,957,125	49,000,000	5,042,875	21
9q12 - q34.3	9	65,632,517	141,213,431	75,580,914	21
14q12-q33.33	14	31,992,119	107,349,540	75,357,421	18
18q11.1-q12.2	18	17,200,000	33,787,598	16,587,598	18
22q13.31 - q13.33	22	45,905,264	50,454,028	4,548,764	18

Figure 2:
Frequency of significant aberrations in primary TCGA samples: State I, II, III versus Stage IV



* Aberrations identified in unmatched dataset occurring significantly (**p-value <=0.05**; Fisher Exact test) higher in TCGA stage IV vs stage I, II, III

Metastatic Site-Specific Aberrations

Table 3:
Significant site-specific aberrations in Lung (n=15) compared with other sites (n=40)

Chr	Start	End	Event	Lung (%)	Other sites (%)
7	0	157,856,060	Gain	53	23
19	26,500,000	43,851,026	Gain	27	5
6	0	61,000,000	Loss	32	5
6	87,071,224	119,699,937	Loss	33	8
10	40,200,000	135,534,747	Loss	27	3

Table 4:
Significant site-specific aberrations in Bone (n=11) compared with other sites (n=44)

Chr	Start	End	Event	Bone (%)	Other sites (%)
12	0	9,108,404	Gain	55	17
12	27,573,538	33,437,301	Gain	55	18

CONCLUSIONS

- 16 significant aberrations were identified to occur at higher frequencies in metastatic compared to primary samples across unmatched and matched analyses
- Among them, gain of 7p and 12q, and loss of 4p, 4q, 9p, 9q, 14q and 18p were significantly occurring higher in stage IV than stage I, II, III in primary TCGA samples
- Upon further validations, in particular prospective studies, such aberrations could serve as biomarkers of metastatic disease and hence could be beneficial in risk-stratification and clinical management of low stage ccRCC patients.
- This preliminary study on identification of metastatic site-specific aberrations could be useful to identify subclinical disease and also helpful in better understanding of the biology of those metastasis

CONFLICTS OF INTEREST

V.T, B.G., and J.H. are full time employees of Cancer Genetics, Inc.

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