Association of Genome-wide Copy Number Alterations with Metastasis of Clear Cell Renal Cell Carcinoma

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OBJECTIVES

➤ To identify genomic copy number alterations (CNAs) associated with metastasis in clear cell renal cell carcinoma (ccRCC) and elucidate their effect on overall survival (OS).

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 a series of copy number changes[4,5], but genomic signatures associated with metastasis/relapse and site of metastasis are not clearly understood.

➤ Identification of novel genomic copy number changes would be beneficial in selecting metastatic patients for appropriate therapy.

MATERIALS & METHODS

➤ Surgically resected primary and metastatic ccRCC (unmatched, fresh frozen) specimens (n=144) were acquired from Memorial Sloan-Kettering Cancer Center for this IRB-approved study (MSK-144 dataset).

➤ Specimen characteristics of the cohort are provided below:

Primary (n=81)

Clinical Stage	No. of specimens
Stage I-III	29
Stage IV	30
Unknown	22

Metastatic (n=63)

Site of Metastasis	No. of specimens
Lung	15
Bone	11
Other	29
Unknown	8

MATERIALS & METHODS

➤ Whole genome array comparative genomic hybridization (aCGH) was performed on DNA extracted from specimens in MSK-144 using 244K array (Agilent Technologies).

➤ Segments defined by Rank Segmentation algorithm using Nexus Copy Number 7.5 (BioDiscovery Inc.)

➤ Minimum average log ratio of 0.13 for gain and -0.13 for loss.

➤ Significantly enriched CNAs were determined between groups using Fisher exact test (P<0.05) and at 15% (primary-metastatic CNAs) and 25% (site-enriched CNAs) differential frequency thresholds.

➤ Effect on OS was estimated by Kaplan Meier method and compared between groups using log-rank test.

STUDY DESIGN

Primary-Metastatic CNAs:

➤ Identification of significantly enriched CNAs between:
i) 81 Primary (All Stages) vs 63 Metastatic
ii) 29 Primary (Stage I-III) vs 63 Metastatic

➤ Verification of frequency of enriched CNAs in Stage I and Stage IV of TCGA-437 and UT-240 primary ccRCC datasets.

➤ Effect of significantly enriched metastatic CNAs on OS in locally-confined (Stages I-III) lesions of TCGA-437 & UT-240.

Site-Enriched CNAs:

➤ Identification of significantly enriched CNAs between:
i) 25 Lung vs 40 Other Sites
ii) 11 Bone vs 44 Other Sites

➤ Verification of frequency of enriched CNAs in primary ccRCC metastasized to either lung/bone versus other sites in UT-240 dataset.

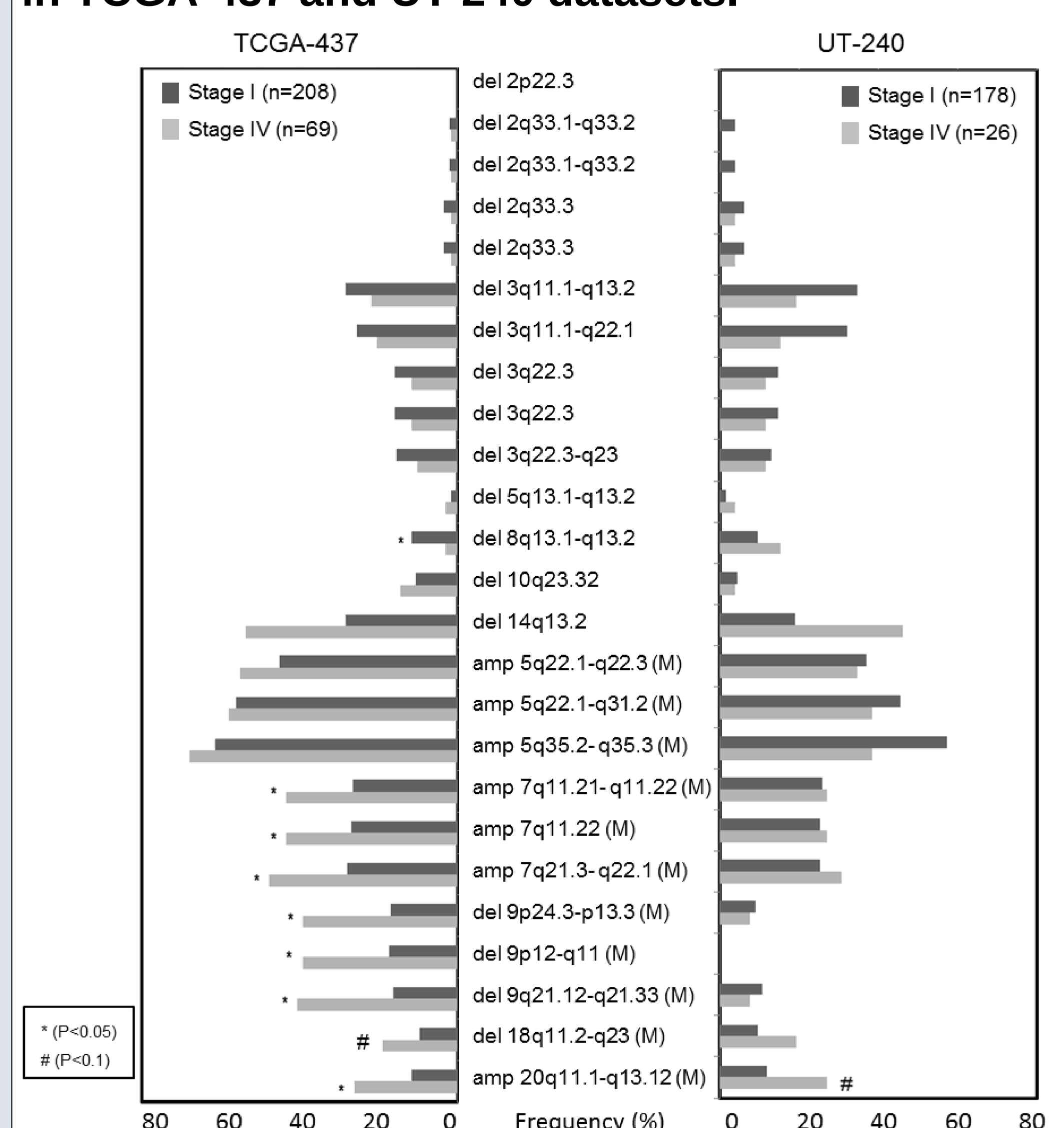
RESULTS: Primary-Metastatic CNAs

Table 1. Primary-metastatic CNAs in MSK-144 dataset.

Aberration	Genomic Coordinates	Primary (%)*	Metastatic (%)*
81 Primary (All Stages) vs 63 Metastatic			
del 2p22.3	chr2: 32,189,545-32,610,016	24.63	7.94
del 2q33.1-q33.2	chr2: 203,011,138-204,488,652	26.96	6.35
del 2q33.3	chr2: 206,878,470-206,898,816	18.52	3.17
del 3q11.1-q13.2	chr3: 91,000,000-111,411,671	23.21	5.49
del 3q22.3	chr3: 135,749,215-136,620,779	27.69	4.67
del 5q13.1-q13.2	chr5: 68,318,311-68,777,505	23.09	6.30
amp 5q22.1-q31.2	chr5: 111,088,102-137,658,174	38.00	56.00
amp 5q35.2-q35.3	chr5: 175,246,582-180,648,778	44.48	62.23
amp 7q11.21-q11.22	chr7: 63,307,561-69,004,244	15.82	31.75
amp 7q11.22	chr7: 70,608,986-71,446,559	16.05	31.75
amp 7q21.3-q22.1	chr7: 97,875,840-98,658,621	20.90	38.10
del 9p24.3-p13.3	chr9: 1-33,816,201	25.60	43.70
del 9p12-q11	chr9: 42,710,743-49,000,000	23.79	40.64
del 9q21.12-q21.33	chr9: 73,749,752-90,080,594	24.69	41.27
del 18q11.2-q23	chr18: 19,406,882-78,077,248	15.11	32.27
amp 20q11.1-q13.12	chr20: 27,500,000-45,248,483	17.00	35.40
29 Primary (Stage I-III) vs 63 Metastatic			
del 2q33.1-q33.2	chr2: 203,061,477-204,563,938	23.97	6.19
del 2q33.3	chr2: 206,871,610-207,136,765	23.21	4.60
del 3q11.1-q22.1	chr3: 91,000,000-130,624,412	24.70	3.56
del 3q22.3	chr3: 135,726,356-136,588,904	26.91	4.61
del 3q22.3-q23	chr3: 138,386,397-138,747,680	17.24	1.59
del 8q13.1-q13.2	chr8: 67,582,497-68,022,127	24.14	7.94
del 10q23.32	chr10: 93,663,791-93,706,767	37.93	17.46
del 14q13.2	chr14: 35,680,178-35,768,604	65.52	41.27
amp 5q22.1-q22.3	chr5: 111,088,102-113,129,196	27.59	51.60

* Weighted Average Frequency

Figure 1. Verification of primary-metastatic CNAs in TCGA-437 and UT-240 datasets.



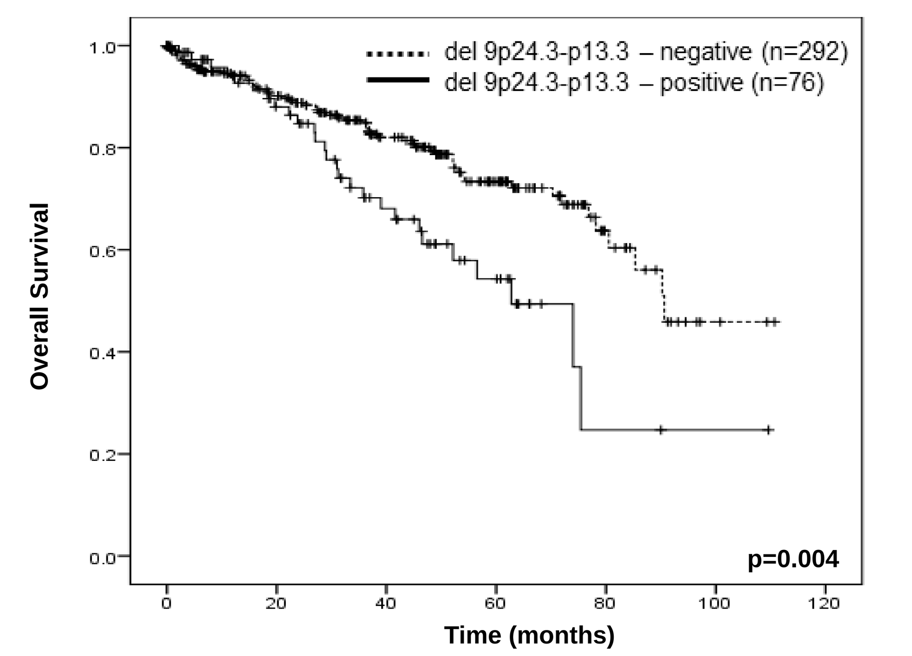
RESULTS: Primary-Metastatic CNAs

Table 2. Effect of metastatic CNAs on OS.

Aberration	Kaplan-Meier Log Rank Test (P value^)	
	TCGA-437 (n=368)	UT-240 (n=214)
amp 7q11.21-q11.22	0.52	0.74
amp 7q11.22	0.38	0.73
amp 7q21.3-q22.1	0.86	0.73
del 9p24.3-p13.3	0.004	0.18
del 9p12-q11	0.007	---*
del 9q21.12-q21.33	0.011	0.26
del 18q11.2-q23	0.913	0.025
amp 20q11.1-q13.12	0.338	0.16

^ statistically significant P-values are highlighted in red
*--- none of the samples were positive for del-9p12-q11

Figure 2. Kaplan-Meier plot for del 9p24.3-p13.3 in Stage I-III of TCGA-437 (n=368).



RESULTS: Site-Enriched CNAs

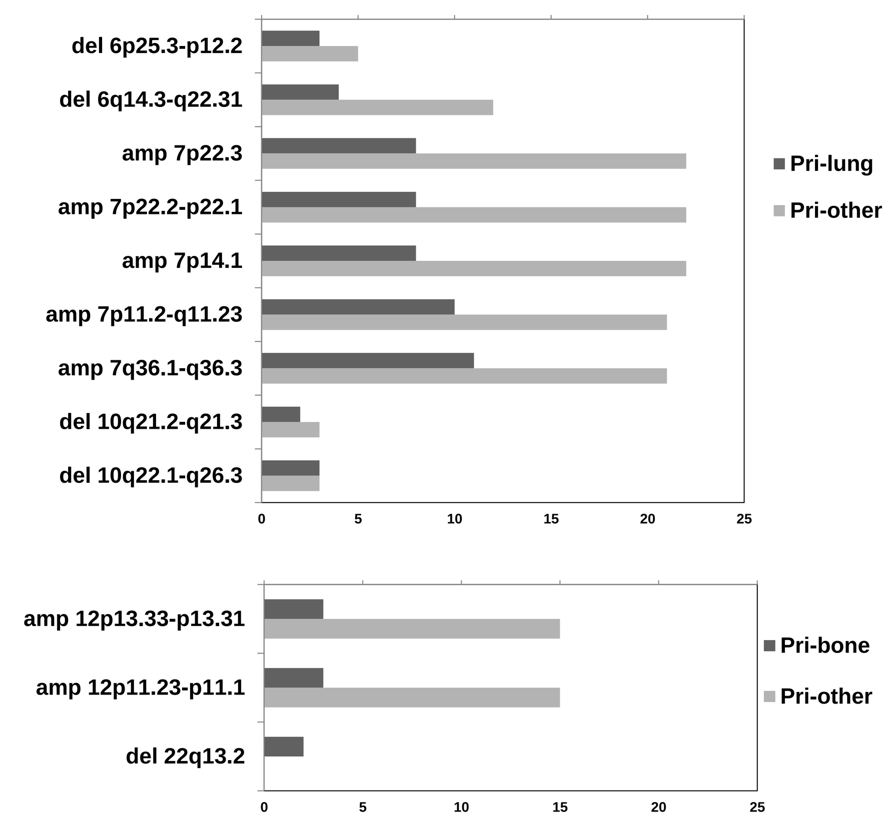
Table 3. Site-enriched CNAs in MSK-144 dataset.

Aberration	Genomic Coordinates	Lung/Bone (%)*	Other (%)*
Lung-enriched CNAs			
del 6p25.3-p12.2	chr6: 1-52,063,841	33.3	5
del 6q14.3-q22.31	chr6: 87,071,224-119,699,937	33.3	7.5
amp 7p22.3	chr7: 1-2,519,207	53.3	22.5
amp 7p22.2-p21.2	chr7: 3,980,964-15,491,937	53.3	18.3
amp 7p14.1	chr7: 39,558,094-40,410,895	53.3	21.3
amp 7p11.2-q11.23	chr7: 57,412,972-76,784,379	53.3	17.4
amp 7q36.1-q36.3	chr7: 148,529,712-157,856,060	53.3	22.4
del 10q21.2-q21.3	chr10: 63,150,893-69,552,141	33.3	2.5
del 10q22.1-q26.3	chr10: 71,052,098-135,534,747	45.9	7.6
Bone-enriched CNAs			
amp 12p13.33-p13.31	chr12: 1-9,108,404	54.8	17.2
amp 12p11.23-p11.1	chr12: 27,573,538-33,437,301	54.5	18.2
del 22q13.2	chr22: 42,189,008-42,419,424	36.4	7.9

* Weighted Average Frequency

RESULTS: Site-Enriched CNAs

Figure 3. Verification of site-enriched CNAs in UT-240 dataset (n=127) with known metastatic status at 5 years after diagnosis.



CONCLUSIONS

➤ A set of 25 significant CNAs identified between primary and metastatic lesions; Among these 25 CNAs, 7 were validated in independent dataset and 4 associated with OS.

➤ Across metastatic lesions, 9 and 3 CNAs were found to be enriched in lung and bone sites respectively.

➤ Genomic signatures identified in this study has the potential to identify patients with high risk of metastasis.

CONFLICTS OF INTEREST

B.G., V.T., C.M., A.G. and J.H. are full time employees and stock/stock option holders of Cancer Genetics, Inc.

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