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

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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% surviving within 5 years of nephrectomy.
- Also, metastatic ccRCC is largely refractory to conventional treatments with a five-year survival rate of 8-10%. Targeted therapies have proven to increase the overall survival rate and treatment options vary depending on the site of neoplasms.
- ccRCC is characterized by a series of genetic alterations that could be utilized for improving diagnosis and prognosis.

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

<table>
<thead>
<tr>
<th>Primary (n=1)</th>
<th>Metastatic (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmatched</td>
<td>Matched Pairs</td>
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- Site of Metastasis (n=10):
  - Lung: N/A
  - Brain: N/A
  - Other: N/A
  - Unknown: 8

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

METHODS

- Whole Genome Array-CGH:
  - DNA extraction resulted in yields >90ug 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)

- 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 (%D)
  - 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:
      - Primary Vs 63 Metastatic
    - Matched Pairs:
      - Primary Vs 34 Metastatic

- Validation of Metastatic Markers:
  - Stage I & II Primary ccRCC (TCGA Dataset) (n=38) :
  - Stage IV Primary ccRCC (TCGA Dataset) (n=98):
  - Differential significance evaluated using Fisher exact test with P-value <0.05

- Analysis of Site-specific Markers:
  - 15 Lung Metastastic Vs 40 Other sites
  - 11 Bone Metastatic Vs 44 Other sites

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

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

- Abbreviations:
  - %:
  - N:
  - Diff.:
  - Freq:
  - Gain:

- Cytoband Chr Start End Event Lung (%)
- Other sites (%)
- Bone (%)
- Other sites (%)

- Table 1: Aberrations occurring significantly higher in unmatched metastatic vs primary.
- Table 2: Aberrations occurring significantly higher in matched metastatic vs primary.
- Table 3: Significant site-specific aberrations in Lung (n=5) compared with other sites.
- Table 4: Significant site-specific aberrations in Bone (n=1) compared with other sites.

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 5p and 10a, and loss of 4q, 4p, 9q, 9p, 14q and 18p were significantly occurring higher in stage IV than stage I, II 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 these metastases.

REFERENCES


- CONFLICTS OF INTEREST

- V.K.B.C., and L.A. are the inventors of a patent at Cancer Genetics, Inc.