Molecular profiling of small cell bladder cancer (SCBC) to reveal gene expression determinants of an aggressive phenotype

Vadim S Koshkin1, Jordan Reynolds1, Paul Elson1, Cristina Magi-Galluzzi1, Jesse McKenney1, Karen S Smith1, Bonnie Shadrach1, Kumiko Issé2, Laura R Saunders2, Ming Hu1, Rahul D Tendulkar1, Andrew J Stephenson1, Ann F Fergany1, Mohamed Abazeed1, Brian I Rini1, Jorge A Garcia1, Byron Lee1, Omar Y Mian1, Petros Grivas1
1. Cleveland Clinic, Cleveland, OH 2. AbbVie Stemcentrx, San Francisco, CA

Small Cell Bladder Cancer (SCBC) is a rare subtype representing about 1% of bladder cancers which has a more aggressive clinical course and worse outcomes compared to urothelial carcinoma.

The data on treatment and outcomes of SCBC is limited and treatment patterns are often extrapolated from small cell lung cancer (SCLC) and urothelial carcinoma.

Biology of SCBC is poorly understood. There have been limited reports on tumor markers and genomic profiling in SCBC, such as somatic alterations linked to treatment response (Teo et al. 2013), which has shown significant anti-tumor efficacy in a Phase I trial in SCLC.

Programming death-ligand 1 (PD-L1) is an important immune checkpoint target by an expanding class of agents, whose expression has not previously been reported in SCBC.

Gene expression profiling of a diverse cohort of SCBC patients allows the classification of these tumors into clusters that correlate with clinical phenotypes.

Differential gene expression analyses compare gene expression of tumor to normal tissue and also among tumor subtypes.

A retrospective review of clinical and pathological characteristics of 63 patients with SCBC seen at Cleveland Clinic from 1990 to 2016 was done following IRB approval.

Small cell histology was confirmed and percentage of small cell component (SCC) was defined in all 63 patient tissues at the time of this analysis by an experienced GI pathologist.

Tumor marker analysis (DLL3 and PD-L1) via IHC and gene expression profiling of a subset of these patients with available tissue specimens was undertaken (Figure 1).

Figure 1: Analysis Schema

Methods

A subset of 53 patients had tissue assessed via IHC for DLL3 expression with anti-DLL3 (IC-16.65) antibody (validated Ventana assay) and PD-L1 (using both SP263 and SP142 antibodies).

Multivariable analyses (MVA) were used to identify patient/tumor characteristics and tumor biomarkers predictive of overall survival (OS), progression-free survival (PFS) and time to progression (TTP) (p ≤ 0.05). Figure 2A: DLL3 Expression: Negative control (top) and tissue with 95% of tumor cells expressing DLL3 (bottom)

Figure 2B: PD-L1 Expression: Negative control (top) and tissue with 5-10% tumor infiltrating cells expressing PD-L1 (bottom)

Results

The majority of SCBC tumors (68%) had DLL3 protein expression, and 22 (35%) / 15 (24%) / 26 (41%) patients were in clusters 1, 2, and 4, respectively.

Unsupervised hierarchical clustering analysis of gene expression in normalized counts per million generated 4 clusters.

Figure 4: Heat map of hierarchical clustering analysis and Kaplan-Meier Curve

Table 1: Clinical/Pathological Characteristics and Survival of SCBC Patients

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>50 (83%)</td>
</tr>
<tr>
<td>Race</td>
<td>49 (78%)</td>
</tr>
<tr>
<td>Age</td>
<td>Median 73</td>
</tr>
<tr>
<td>Carcinoma Stage</td>
<td>T1-2</td>
</tr>
<tr>
<td>Surgery</td>
<td>44 (77%)</td>
</tr>
<tr>
<td>Pathology</td>
<td>23 (36%)</td>
</tr>
<tr>
<td>Prior Radiotherapy</td>
<td>11 (17%)</td>
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<tr>
<td>OS From Diagnosis</td>
<td>6.2 (6.0-11.2)</td>
</tr>
<tr>
<td>PFS Based on Small Cell %</td>
<td>5.3 (4.6-6.7)</td>
</tr>
</tbody>
</table>

Figure 5: Differential gene expression analysis among the 4 clusters

Conclusions

Unsupervised hierarchical clustering of gene expression patterns from a heterogeneous cohort of small cell bladder cancer patients produced 4 distinct gene expression clusters that correlated with clinical phenotypes.

This is the first study to reveal distinct gene expression patterns in SCBC that define aggressive behavior and are associated with worse clinical outcomes including shorter OS and PFS.

DLL3 gene expression had a strong correlation with DLL3 protein expression suggesting its regulation at the transcriptional level.

The majority of SCBC tumors (68%) had DLL3 protein expression, and 30% had PD-L1 expression.

Higher DLL3 expression and increased small cell component were prognostic of worse clinical outcomes in SCBC.

Prognostic value of differential gene expression networks and the presence of underlying genomic and epigenetic alterations is the subject of ongoing investigation in this patient cohort.

Please read correspondence to Vadim Koshkin (vdos@ccf.org)