Molecular Profiling of Small Cell Bladder Cancer (SCBC) Reveals Gene Expression Determinants of an Aggressive Phenotype

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Background:

- SCBC is a rare variant of bladder cancer with relatively poor outcomes.
- Treatment options are often extrapolated from other anatomic sites.
- We performed molecular profiling to characterize unique features in SCBC with the goal of identifying novel therapeutic targets and biomarkers for risk stratification.

Methods:

- A retrospective analysis of 63 patients with biopsy-confirmed SCBC treated with radiotherapy, chemotherapy, and surgery at a single high volume center between 1994-2015.
- Small cell histology (SC%) was quantitatively determined by independent pathologic review. DLL3 and PD-L1 protein expression were measured by IHC in 53 patients.

Figure 1: DLL3 and PDL1 Immunohistochemistry in 53 Patients with Available Tissue

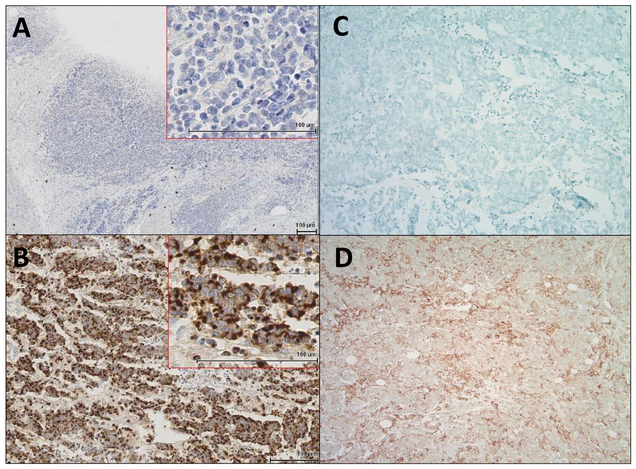


Figure 1 Immunohistochemistry: DLL3 Expression, (A) Negative control and (B) tissue with 95% of tumor cells expressing DLL3 (B). PDL1 Expression, (C) Negative control and (D) tissue with 5-10% tumor infiltrating cells expressing PD-L1.

- Formalin fixed and paraffin embedded tumor tissue was available for gene expression analysis in 39/63 primary tumors (median SC%=100%), 1 metastatic sample, and 6 adjacent normal samples (n=46 total) using the HTG EdgeSeq OBP assay with probes for 2568 genes.
- An average of 1.1x107mRNA probes were sequenced per sample and analyzed using the RNAseq workflow in the Partek Genomics Suite.

Results:

Figure 2: Baseline Patient Characteristics and Independent Prognostic Factors of OS, PFS and TTP

Variable	All Patients (N = 63)	Cystectomy Patients (N = 41)
Median Age	71 (39-90)	70 (39-87)
ECOG PS at Diagnosis 0 1 2 3 Unknown	 22 (56%) 10 (26%) 4 (10%) 3 (8%) 24	 17 (65%) 7 (27%) 2 (8%) 0 15
Gender Male Female	 52 (83%) 11 (17%)	 35 (85%) 6 (15%)
Current/Former Smoker Yes No	 44 (77%) 19 (23%)	 30 (81%) 7 (19%)
Hematuria at Presentation Yes No	 56 (89%) 7 (11%)	 36 (88%) 5 (12%)
Hydronephrosis Yes No	 9 (15%) 54 (85%)	 5 (13%) 35 (87%)

Overall Survival (OS)		
Factor	HR (95% CI)	p
% cells (DLL3+) (>10% vs ≤10%)	**2.8** (1.1-6.7)	.03
pT Stage* (T3/T4 vs T1/T2)	**2.5** (1.0-6.2)	.05

Progression-Free Survival (PFS)		
Factor	HR (95% CI)	p
% cells (DLL3+) (>10% vs ≤10%)	**2.5** (1.1-5.8)	.04
Small Cell %* (>50% vs ≤ 50%)	**3.6** (1.2-10.9)	.02
Margins* (positive v negative)	**2.3** (1.0-5.3)	.06

Time to Progression (TTP)		
Factor	HR (95% CI)	p
% small cell* (>50% vs ≤50%)	**4.0** (1.1-14.4)	.03

Pathologic Features of Cystectomy Patients	N = 41
Tumor Location	Bladder: 37 (90%) Bladder and Ureter: 2 (5%) Bladder and Urethra: 1 (2%) No tumor (T0): 1 (2%)
Pathologic T-stage	T0-T2: 15 (37%) T3-T4: 26 (63%)
Pathologic N-stage	N0: 23 (56%) N1-N3: 18 (44%)
Surgical Margins	Positive: 8 (22%) Negative: 29 (78%)
Carcinoma in Situ (CIS)	Present: 24 (60%) Absent: 16 (40%)

*pT Stage, Small cell % and Margin status outcomes measured from time of cystectomy

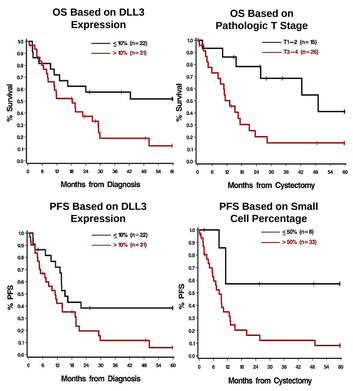


Figure 3: Gene Expression Clustering and Survival Analysis by Cluster

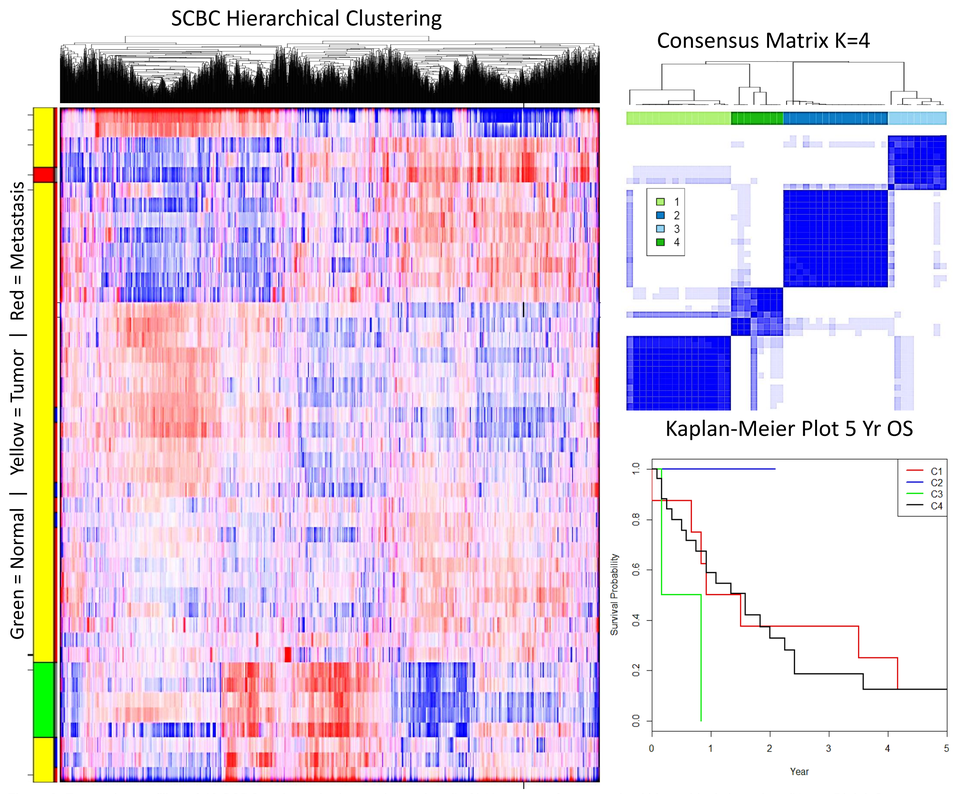
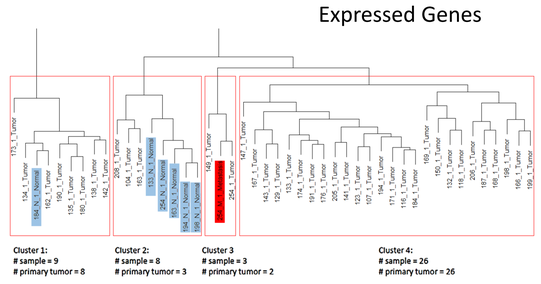


Figure 3: Expression profiling of 46 SCBC and matched normal samples, (Left) Heat map of unsupervised hierarchical clustering. (Upper Right) Consensus clustering demonstrates 4 stable clusters. (Lower Right) Kaplan Meier survival demonstrates significant a differences in survival between clusters.

Figure 4: Dendrogram of Gene Expression Clusters and Top Differentially Expressed Genes



Gene	ANOVA_P value
TNFAIP3	1.26E-15
DUSP1	3.54E-14
ADAMTS1	1.13E-13
GADD45B	1.92E-13
IER3	3.03E-13
SOCS3	3.10E-13
DUSP5	1.82E-12
PABP1	3.11E-12
SGK1	4.58E-12
CD44	6.37E-12
SOD2	7.96E-12
UBE2T	8.08E-12
LIF	1.23E-11
ICAM1	1.38E-11
EMP1	1.41E-11
BIRC3	1.67E-11
CCT2	1.74E-11
KPNA2	2.70E-11
IL1R1	3.13E-11
FOS	3.14E-11

Figure 4: (Left) Clustering dendrogram demonstrating 4 primary clusters. (Right) Top differentially expressed genes by cluster.

Figure 5: Gene Expression Principle Components Analysis

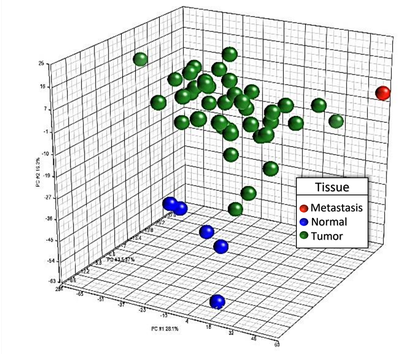


Figure 5: PCA analysis of gene expression data demonstrating separation between normal, primary tumor and metastatic samples

Figure 6: EZH2 Expression

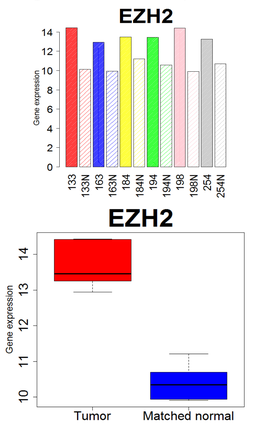
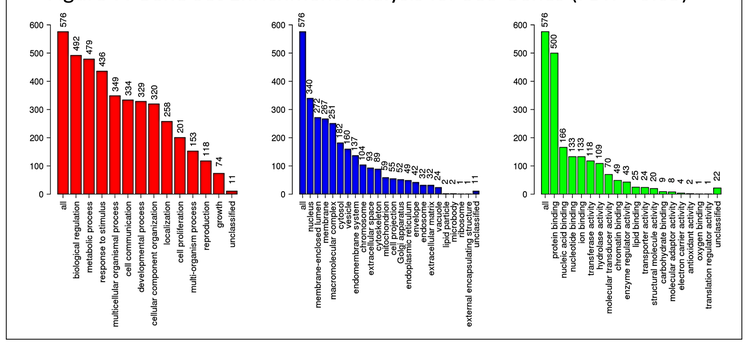


Figure 6: EZH2 overexpressed in tumor vs adj. normal

Figure 7: Gene Set Enrichment Analysis for 583 Genes (FDR < 0.05)



Conclusions:

- A distinct gene expression signature defines aggressive behavior, metastatic potential and outcomes in SCBC.
- DLL3 is transcriptionally regulated in SCBC and is associated with survival
- EZH2 is overexpressed in SCBC vs adjacent matched normal urothelium
- The prognostic value of differential gene expression networks and the presence of underlying genetic and epigenetic alterations is the subject of ongoing prospective validation in a larger cohort.

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