A multiplatform biomarker analysis of small cell bladder cancers

Jue Wang[1], David Arguello[2], Sandeep Reddy[2], Zoran Gatalica[2]
[1]University of Arizona Cancer Center at St. Joseph's Medical Center, Phoenix, AZ; [2]Caris Life Sciences, Phoenix, AZ

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Abstract

Background: Small cell bladder cancer (SCBC), a malignancy indistinguishable from small cell lung cancer (SCLC), is a rare and aggressive subtype of bladder cancer. Response to chemotherapy in SCBC is poor, yet the standard therapy remains cisplatin and etoposide. Novel therapies based on a better understanding of the underlying mechanisms of transformation are needed. The purpose of this study is to identify potential targets and therapeutic options for this disease, using multiplatform tumor profiling.
Methods: In total, 19 small cell bladder cancer specimens were tested via a multiplatform profiling service (Caris Life Sciences, Phoenix, AZ) consisting of gene sequencing (Sanger or next generation sequencing [NGS]), protein expression (immunohistochemistry [IHC]) and gene amplification (CISH or FISH).
Results: Loss of RRM1 (22.2%, 4/18), MGMT (83.3%, 15/18), and TS (26.3%, 5/19) by IHC are associated with potential benefit to traditional chemotherapy. High expression of ERCC1, associated with resistance to platinum-based therapy, was 37.5% (3/8). High expression of TOP2A (93.8%, 15/16) and TOPO1 (78.9%, 15/19) are associated with treatment benefit to anthracyclines and camptothecins, respectively. MRP1, a drug pump associated with resistance to various chemotherapies, was present in 100% (5/5) of specimens. PD-L1 (0%, 0/6) was not expressed. EGFR amplification was detected in 25.0% of patients (1/4). NGS aberrations included TP53 (90.0%), cMET (20.0%, 2/10), RB1 (11.1%), FBXW7 (10%, 1/10), PTEN (10%, 1/10). Sanger sequencing also detected KRAS (100%, 1/1) and PIK3CA (33.3%, 1/3) mutations.
Conclusion: Multiplatform tumor profiling may identify candidates of agents to be tested in prospective clinical trials. Biomarker results like MRP1 overexpression may explain this cancer's resistance to traditional chemotherapy and its aggressive course. NGS and Sanger sequencing identified cell surface receptors and downstream molecules that could be candidates for therapeutic strategies.

Background

Bladder cancer is one of the most common malignancies, with urothelial being the most common subtype. Small cell bladder cancer (SCBC), a malignancy indistinguishable from small cell lung cancer (SCLC), is a rare and aggressive subtype of bladder cancer, comprising

Background (cont.)

only 0.5-1.0% of bladder malignancies. In fact, up to 65% have metastases at, or soon after, diagnosis. The standard therapy for SCBC is cisplatin and etoposide, however, response to chemotherapy is poor. Novel therapies based on a better understanding of the underlying mechanisms of transformation are needed. The purpose of this study is to identify potential targets and therapeutic options for this disease, using multiplatform tumor profiling.

Methods

In total, 19 small cell bladder cancer specimens were tested using a CLIA-certified, multiplatform profiling service (Caris Life Sciences, Phoenix, AZ). Protein expression was assessed by immunohistochemistry (IHC). Gene amplification was determined using fluorescent in situ hybridization (FISH) or chromogenic in situ hybridization (CISH). Sequencing was performed by either Sanger or next generation sequencing (NGS), with NGS evaluating up to 47 genes at a depth of 1500x.

Results

Nineteen SCBC specimens were tested . The average age of this cohort was 67.6 years old. Most patients were male (see Figure 1).

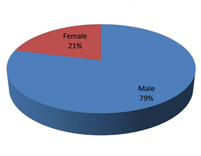


Figure 1

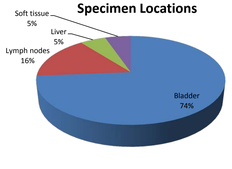


Figure 2

Most specimens (see Figure 2) were collected from the bladder using transurethral resection of the bladder (TURB). The second most common collection site was lymph nodes, either pelvic (n=2) or supraclavicular (n=1).

Results (cont.)

The bar chart (Figure 3) below shows distribution of IHC in SCBC.

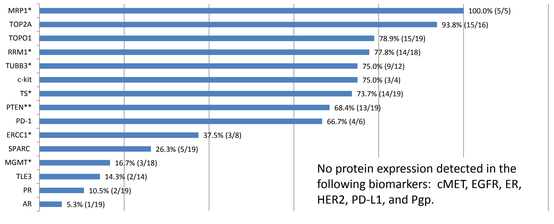
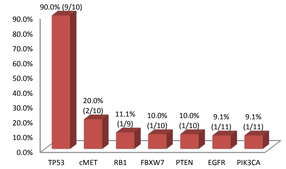


Figure 3

The high expression of MRP1 may explain why cytotoxic therapy fails in this disease. In addition, high expression of RRM1, TUBB3, and TS imply resistance to agents such as gemcitabine, paclitaxel, and fluorouracil. Regardless, high protein expression of TOP2A, TOPO1 indicate anthracyclines (i.e. doxorubicin) and camptothecins (i.e. irinotecan) may play a role in disease management. MGMT may identify those patients who derive benefit from temozolomide.

Mutations detected by NGS or Sanger sequencing revealed the following (see Figure 4):



No mutations were detected in the following biomarkers: ABL, AKT1, ALK, APC, ATM, BRAF, BRCA1, BRCA2, CDH1, cKIT, CSF1R, CTNNB1, ERBB2, ERBB4, FGFR1, FGFR2, FLT3, GNA11, GNAQ, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR, KRAS, MLH1, MPL, NRAS, PDGFRA, PTPN11, RET, SMAD4, SMARCB1, SMO, STK11, and VHL.

Figure 4

Like small cell lung cancer, TP53 was the most common mutation (90.0%). The presence of PIK3CA, PTEN, and FBXW7 mutations warrants consideration of targeting the PIK3CA/Akt/mTOR pathway in a subset of patients. Targeting cMET may be an option in a small group of these patients as well, although the utility of cMET mutational screening is still under debate. The lack of mutations in this disease warrants further studies involving multi-omic modalities to better understand and target this disease.

Select Mutations in SCBC	
Gene	**Protein Change**
EGFR	G719S
FBXW7	T576M
PIK3CA	E542K
PTEN	L247fs
TP53	G165X, Q192fs, R213L*, Y236C, R248W, R273C, D281N, E285K, E339X

*G to T transversion

Figure 5

The table on the left (Figure 5) shows mutations detected by sequencing. TP53 mutations (66.7% missense, 22.2% nonsense, 11.1% frameshift) changing the coding sequence were dispersed between amino acids 165 – 339 (i.e. exons 5 – 10). Unlike SCLC, G to T transversions were not commonly observed (11.1%, 1/9).

ISH	Amplified	Total	Percent
EGFR	1	4	25%
cMET	0	8	0%
HER2	0	15	0%
TOP2A	0	3	0%

Figure 6

Other than EGFR in a single case, amplification was not detected in the cohort (see Figure 6). Combined with the EGFR mutation detected, a small group of SCBC patients may derive benefit from EGFR-targeted therapy or therapy directed downstream of EGFR.

Conclusions

- To the best of our knowledge, this is the most comprehensive molecular analysis of SCBC, a malignancy accounting for 1% of all bladder cancers.
- High IHC expression of biomarkers such as MRP1, RRM1, TUBB3, and TS may contribute to this malignancy's resistance to cytotoxic chemotherapy.
- Taking the combination of low ERCC1 and high TOP2A expression by IHC into account may identify those patients who derive the most benefit from upfront cisplatin plus a TOP2A inhibitor. In addition, low or absent MGMT protein expression may identify those patients who derive benefit from temozolomide. Prospective trials are needed to test the potential value of these biomarkers for response.
- Similar to small cell lung cancer (SCLC), TP53 mutations are found in SCBC with high frequency.
- Based on sequencing results, other potential options in this disease include employing agents targeting the PIK3CA/Akt/mTOR therapy.
- A small group of SCBC have amplified EGFR or EGFR mutations. Clinical trials testing efficacy of EGFR inhibition may be worthwhile.
- A multiplatform approach may be valuable for identifying novel targets in this rare disease. Larger analyses of this aggressive disease is warranted.

References

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