Commentary

Comprehensive Genomic Characterization of Small Cell Bladder Cancers Reveals Novel Therapeutic Targets

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Background

Small cell bladder cancer (SCBC) is a rare and aggressive subtype of bladder cancer, comprising only 0.5-1.0% of bladder malignancies. Histology and immunohistochemistry shows a tumor which is indistinguishable from small cell lung carcinoma (SCLC). Previously we have shown that SCBC is predominantly a disease of elderly patients, who present with stage IV cancer at the time of diagnosis in up to 50% cases [2]. SCBC is one of the most aggressive and fast growing slid tumor with median survival only 11 months [1]. The treatment is extrapolated from that of SCLC. In surgically resectable disease, multimodal therapy with chemotherapy first followed by radical resection or radiotherapy. In advanced disease, chemotherapy using platinum agent (cisplatin in patients with good kidney function) is the mainstay treatment. However, response to chemotherapy is not ideal and not durable. Novel therapies based on a better understanding of the underlying mechanisms of transformation and chemoresistance are needed.

Identification Molecular Alterations in SCBC

Molecular data regarding urinary bladder SCC are scarce in comparison to conventional urothelial carcinoma [3]. Chang et. al reported that small cell cancers of the bladder and lung have a convergent but distinct pathogenesis with SCBC arising from a cell of origin shared with urothelial bladder cancer [4]. Recently, we performed comprehensive integrated genomic analyses to further characterized the genomic landscape of SCBC (8). Nineteen 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 sequence.

The average age of our study cohort was 67.6 years old. Most patients were male. Most specimens were collected from transurethral resection of the bladder (TURB). The second most common site was metastatic lymph nodes. Similar to small cell lung cancer, TP53 was the most common gene mutation detected by NGS (90.0%). Followed by c-MET (20.0%, 2/10), RB1 (11.1%), FBXW7 (10%, 1/10), PTEN (10%, 1/10). The majority of TP53 mutations are single base-pair substitutions. Among the TP53 mutations identified, 66.7% were missense, 22.2% were nonsense, 11.1% were frameshift. TP53 hot spot mutations 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). Sanger sequencing also detected KRAS (100%, 1/1) and PIK3CA (33.3%, 1/3) mutations. Loss of RRM1 (22.2%, 4/18), MGMT (83.3%, 15/18), and TS (26.3%, 5/19) were detected by IHC. High expression of ERCC1 was found in 37.5% (3/8) samples. High expression of TOP2A was found in 93.8% (15/16) and TOPO1 in 78.9% (15/19) of specimens. MRP1 was present in 100% (5/5) of specimens. PD-L1expression was not detected (0%, 0/6). EGFR amplification was detected in 25.0% of specimens (1/4).

Clinical Implications of these Findings

Comparison of mutational profiles of urothelial carcinoma and small cell carcinoma revealed a significantly higher incidence of mutations in components of p53 signaling and greater genomic instability.

Our findings of 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 chemotherapeutic agents such as gemcitabine, paclitaxel, and fluorouracil. On the other hand, high protein expression of TOP2A, TOPO1 indicate anthracyclines (i.e. doxorubicin) and camptothecins (i.e. irinotecan) may be worthy for further investigation in this disease.

One particular notable finding in this study is a significant number of patients had low or absent MGMT protein expression in our

cohort of SCBC patients, which indicates a potential benefit with temozolomide, a chemotherapeutic agent currently approved for the treatment of glioblastoma multiform [5] and melanoma [6]. In colorectal cancer, patients whose tumors expressed low or undetectable levels of MGMT protein had a better outcomes following TMZ treatment than their counterparts [7].

Limitations

Our genomic analyses of nineteen SCBC specimens have several limitations. The lack of detail clinicopathologic information limited our ability to further identify groups of SCBC patients who may derive more benefit from certain chemotherapeutic or targeted therapies. Regardless, however, given the rarity of these tumors, our evaluation also adds to the knowledgebase of this rare aggressive bladder cancer, both from a genomic profile perspective as well as identification of potential targeted therapies. The overexpression of several proteins, as identified by IHC, further advances the understanding of why the clinical benefit of cytotoxic chemotherapies is usually short-lived in the treatment of this disease.

Future Perspective

The results from ours study and others have shown that 90% of SCBC tumors harbor mutations in the TP53 tumor suppressor gene [3,4,8], other studies demonstrated that disruptive mutations in TP53 are associated with higher tumor grade and advanced stage, as well as worse prognosis and survival. Mutations are primarily localized to the highly conserved DNA binding domain and inactivate wild type (WT) p53 function. TP53 alterations alone cannot provide definitive information regarding its function in the setting of SCBC. As each p53 mutation may have unique properties, future studies in SCBC animal models is critical to understand how these alterations affect biology of SCBC and the emerging of chemoresistance.

Since TP53 is recognized as a master transcription factor and guardian of the genome that regulates the expression of a plethora

of genes involved in crucial biological processes, we hypothesized that TP53 loss may confer dominant malignant phenotypes in SCBC cancer cells also engender unique, exploitable vulnerabilities. Since p53 mutant SCBC are aggressive tumors incapable of G1 arrest and with higher levels of genomic instability, these tumor cells rely on a functional G2/M cell cycle checkpoint to repair the DNA damage that might occur as a result of genomic instability or through genotoxic chemotherapy. Despite overwhelming evidence implicating the role of p53 functional derangement in the tumor biology and clinical outcome of bladder cancer [3,4,8-10], there were no targeted therapies that capitalize on this knowledge till researchers identified p53 synthetic lethal interactions with several G2/M checkpoint regulators. WEE1 is a tyrosine kinase that phosphorylates and inhibits cyclin-dependent kinases 1 (CDK1) and 2 (CDK2), and is involved in regulation of the intra-S and G2 cell cycle checkpoints. AZD1775, an inhibitor of WEE1, is currently in phase II trial (NCT02688907) as monotherapy for relapse small cell lung cancer patients. To translate these findings to the clinic, clinical trial with AZD1775 monotherapy or in combination with chemotherapy should be tested in metastatic SCBC patients.

Immune checkpoint inhibitors have evolved as a critical component of the treatment of multiple tumor entities including bladder and non-small cell lung cancer. Recently, the FDA has granted an accelerated approval to checkpoint inhibitor nivolumab (Opdivo) for the treatment of patients with small cell lung cancer (SCLC) with disease progression following platinum-based chemotherapy and 1 other line of therapy. There cases reports indicating checkpoint inhibitor is active against SCBC with high tumor mutation burden. Currently available biomarkers such as PD-L1 expression, tumor mutation load (TML), microsatellite instability (MSI)/mismatch repair deficient (dMMR) status, and mutational burden show incomplete predictive performance. Prospective study the efficacy of these agents in patients is still warranted despite of the findings of PD-L1expression in our small cohort.

For disease such as SCBC, understanding underlying mechanisms of acquired treatment resistance is just as critical as comprehending mechanisms that confer susceptibility to therapy. In order to further understand these mechanisms, re-biopsy recurrent disease sites at progression is very important but at the same time may be difficult to be obtained. Detection of circulating tumor DNA in the plasma of patients with cancer, the so-called 'liquid biopsy', represents an exciting field of investigation also in bladder cancer [11,12], where the serial monitoring of circulating tumor DNA can reveal altered prevalence of mutations in patients with emerging resistance to therapies. Our finding suggests tracking the mutation changes during chemotherapy may provide critical insight to address chemoresistance.

Conclusions

Precision medicine aims to identify novel targets for treating rare and difficult-to-treat cancers such as SCBC. Our recent high-throughput comprehensive genomic characterization profiling of SCBC and circulating tumor (ct)-DNA studies addresses one of the most urgent needs in the clinical management of this aggressive disease, the development of more effective, targeted and less toxic therapies. Our study supports a rationale for testing several druggable targets in SCBC patients. Based on this work, a clinical trial has been proposed that will identify and treat the subset of SCBC patients who have the specific targets present in their tumor.

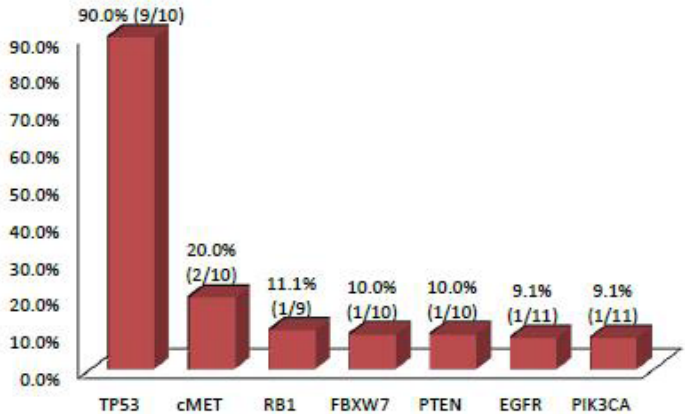

Figure 1: Alteration frequencies of the genes identified in small cell bladder cancer (SCBC) by Next-generation sequencing (NGS) or Sanger sequencing.

Table 1: TP53 mutations and corresponding protein changes in small cell bladder cancer (SCBC) detected by gene sequencing.

Gene	Protein Change
TP53	G165X, Q192fs, R213L*, Y236C, R248W, R273C, D281N, E285K, E339X

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