Molecular profiling of non-urothelial bladder cancer: adenocarcinoma and squamous cell carcinoma

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Abstract#423

Background: Adenocarcinoma (ADA) and squamous cell carcinoma (SCC) are rare and often aggressive histologic subtypes of bladder cancer. For advanced disease, no clear standard therapies exist and NCCN guidelines suggest only fluorouracil, cisplatin, paclitaxel and ifosfamide as possible options. Thus, novel therapies based on underlying tumor biology are needed. The purpose of this study was to identify potential targets and therapeutic options for these histologic subtypes, utilizing multiplatform tumor profiling.

Methods: 49 ADA and 24 SCC specimens were tested via a multiplatform profiling service (Caris Life Sciences, Phoenix, AZ) consisting of gene sequencing (Sanger or next generation sequencing [NGS]), gene amplification (CISH or FISH), and protein expression (immunohistochemistry [IHC]). Tissue from a metastatic site was submitted in 52% of the cases.

Results: Both ADA and SCC exhibited high rates of TP53 aberrations (82.4% and 72.7%, respectively). Sequencing revealed mutations in BRCA2 (14.3%), SMAD4 (12.5%), PTEN (11.8%), KRAS (8.7%), NRAS (5.6%), and KIT (5.3%) in ADA. In addition, PIK3CA (21.4%), HRAS (18.2%), BRCA1 (16.7%), BRCA2 (16.7%), and FBXW7 (9.1%) mutations were detected in SCC. Amplification in EGFR (27.3%) and ERBB2/HER2 (16.7%) were found in ACA. Meanwhile, only one ERBB2 (6.3%) amplification was found in SCC using ISH. MET was not amplified in either ACA or SCC. For both ACA and SCC, EGFR had the highest level of protein expression (100% and 85.7%, respectively). Of note, PD-1 (44.4% in both) and PD-L1 (11.1% and 22.2% in ACA and SCC, respectively) were expressed in both subtypes. Although differential rates of somatic alterations, amplification, and protein expression were found between ADA and SCC, only TLE3 was significant (19.2% versus 60.0%, respectively, p=0.0154).

Conclusion: Differential results in gene alteration, amplification, and protein expression imply the potential utility of tumor profiling in guiding therapeutic decision-making in ADA and SCC of the bladder. Aberrations in the PIK3CA/AKT/mTOR pathway and alterations in TP53 in these subtypes are similar to what has been reported in urothelial bladder cancer. Targeting the PD-1/PD-L1 axis may also be a therapeutic option. Further studies are warranted to better manage these two rare malignancies.

Results

Histology	Number	Median Age (Range)	Male:Female Ratio	Percent Metastatic
Adenocarcinoma	49	62 (37 – 80)	2:1	46.9% (23/49)
SCC	24	60.5 (37-82)	1:1	50.0% (12/24)

Table 1 – Patient Specimen Information. Bladder specimens were from either TURBT or total cystectomy. No formal staging data was available. Roughly half were from distant metastatic sites. Only one adenocarcinoma specimen was confirmed as urachal in origin. No information was available regarding prior history of schistosomiasis in the SCC cohort.

Results (continued)

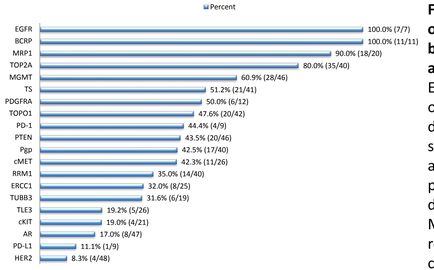

Figure 1 – Protein overexpression of bladder adenocarcinoma. EGFR overexpression was detected in all specimens analyzed. High protein levels of drug pumps (BCRP, MRP1) may explain resistance to conventional therapy. PD-1, a marker for tumor infiltrating lymphocytes (TILs), was 44.4%, with PD-L1 overexpression being detected in 11.1% of specimens analyzed. Other potentially predictive biomarkers are shown. HER2 overexpression was found in 8.3%.

Biomarker	Percent Amplification
EGFR	27.3% (3/11)
ERBB2 (HER2)	16.7% (5/30)
MET	0.0% (0/21)

Table 2 – Gene copy number alterations in bladder adenocarcinoma. *EGFR* amplification was detected in 27.3% of specimens evaluated. In addition, *ERBB2* (*HER2*) amplification was also detected in 16.7% of specimens. Although cMET protein overexpression was detected in a number of specimens (see Figure 1), it was not secondary to *MET* gene copy alterations.

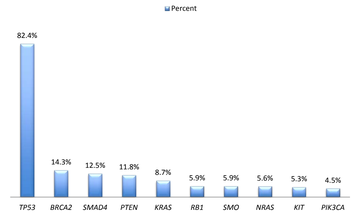

Figure 2 – Sequencing analysis using either Sanger or NGS in bladder adenocarcinoma. A high percentage (82.4%) of TP53 aberrations were found in the adenocarcinoma cohort. Other aberrations were also detected by sequencing, with some being potentially targetable. Of note, PIK3CA alterations were detected in 4.5% of specimens. Most of the 47 genes analyzed in this cohort showed no aberrations. These included the following: *ABL1, AKT1, ALK, APC, ATM, BRAF, BRCA1, CDH1, cMET, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR, MLH1, MPL, NOTCH1, NPM1, PDGFRA, PTPN11, RET, SMARCB1, STK11*, and *VHL*.

Results (continued)

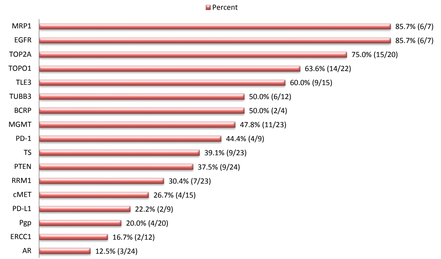

Figure 3 – Protein overexpression of bladder SCC. The drug pump MRP1 showed the highest rate of over-expression. A high rate of EGFR overexpression was also detected in this small cohort. PD-1, a marker for tumor infiltrating lymphocytes (TILs), was 44.4%, the same percentage as in adenocarcinoma. However, PD-L1 overexpression, an emerging biomarker used for predicting response to immune checkpoint inhibitors, was detected in 22.2% of specimens analyzed, double the rate of adenocarcinoma. Other potentially predictive biomarkers are shown. HER2 overexpression was not detected.

Biomarker	Percent Amplification
EGFR	0.0% (0/5)
ERBB2 (HER2)	6.3% (1/16)
MET	0.0% (0/14)

Table 3 – Gene copy number alterations in bladder SCC. In contrast to adenocarcinoma, few amplifications were detected, although this may have been due to the low number of specimens analyzed. As in other histologic subtypes, ERBB2 (HER2) amplification is detected in bladder SCC (6.3%).

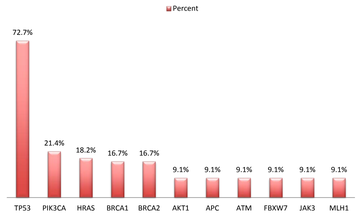

Figure 4 – Sequencing analysis using either Sanger or NGS in bladder SCC. In bladder SCC, high TP53 mutation rates are observed. A closer evaluation reveals dysregulation along the PIK3CA/AKT/mTOR pathway, as evidenced by Relatively high rates of mutations in PIK3CA (21.4%), AKT1 (9.1%), and FBXW7 (9.1%). Other potentially targetable aberrations were also found. Most of the 47 genes analyzed in this cohort showed no aberrations. These included the following: ABL1, ALK, BRAF, CDH1, cKIT, cMET, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HNF1A, IDH1, JAK2, KDR, KRAS, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, AND VHL.

Results (continued)

Biomarker	Bladder Adenocarcinoma	Urothelial Bladder Carcinoma
cMET	42.3%	16.7%
Pgp	42.5%	16.4%
PTEN	43.5%	69.3%
TOPO1	47.6%	70.8%
TP53	82.4%	54.1%
Biomarker	**Bladder SCC**	**Urothelial Bladder Carcinoma**
PTEN	37.5%	69.3%

Table 4 – Comparison of bladder adenocarcinoma and bladder SCC with urothelial carcinomas. This table shows biomarkers that are significantly different ($p < 0.05$) between the non-urothelial carcinoma specimens in the cohort and 221 consecutive urothelial bladder carcinomas culled from the Caris Life Sciences database. Most differences were detected in the IHC panels with the exception of TP53 by NGS in the comparison between bladder adenocarcinoma and urothelial bladder carcinoma. Fewer differences are observed in bladder squamous cell carcinoma, perhaps due to the low overall number of SCC available for comparison.

Conclusions

- Multi-omic profiling can identify differences in the underlying biology of adenocarcinoma and squamous cell carcinoma of the bladder.
- In adenocarcinoma, comparatively high ERBB2 and EGFR should be evaluated further in clinical trials with newer pan-HER therapies given previous negative studies using older HER-targeted therapy.
- The higher rate of dysregulation along the PIK3CA/AKT/mTOR pathway in bladder SCC warrants further investigation.
- Targeting the PD-1/PD-L1 axis in non-urothelial bladder cancer is worthy of clinical trial investigation.
- Future analyses of these malignancies should include emerging markers such as TSC1 and FGFR3.

References

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