Molecular characterization of squamous cell carcinoma of the anal canal (SCCA)

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Abstract #176039

Background: Nivolumab has shown promising results in SCCA patients. The majority of SCCA cases have been linked to prior human papillomavirus (HPV) infection. However, HPV negative tumors are frequently *TP53* mutated and often resistant to therapy. Molecular characteristics of SCCA are largely undefined. Here we explored the underlying biology of SCCA and the differences between *TP53*-wild type (*TP53*-WT) and *TP53*-mutated (*TP53*-MT) tumors.

Methods: SCCA specimens underwent multiplatform testing with protein expression (IHC), gene amplification (ISH), and sequencing (NGS). Tumor mutational burden (TMB) was calculated using only somatic nonsynonymous missense mutations. Chi-square tests were used for comparative analyses.

Results: In total, 253 tumors were studied. The most frequently mutated genes included *PIK3CA* (24%), *BRCA2* (14%), *FBXW7* (12.4%), *TP53* (9.7%), and *PTEN* (8.9%). In a subset of 23 tumors subjected to Illumina NextSeq (592 gene) testing, the most common mutations were *NOTCH2* (30%), *NOTCH1* (27.3%), *POLE* (21.7%), *TSC2* (17.4%), *PTEN* (14.3%), *BRAF* (13.6%), *BRCA2* (13.0%), *PIK3CA* (13.0%), and *FBXW7* (9.5%). Tumors frequently expressed MRP1 (97.6%), EGFR (92.7%), TOP2A (88.5%), TOPO1 (69.5%), MGMT (67.8%), and RRM1 (59.9%). Expression of PD-1 was seen in 55.8% (24/43) of tumors, and PD-L1 in 15.4% (9/34). HER2 was amplified in 2% (3/147) of samples, which has not been previously described in SCCA. When compared with *TP53*-WT (n = 93) tumors, *TP53*-MT (n = 10) had higher rates of *BRAF* (22% vs. 1%, p < 0.001) and *RB1* mutations (44% vs. 0%, p < 0.001), whereas *TP53*-WT had higher expression of TOPO1 (76% vs. 40%, p = 0.01) and TUBB3 (19% vs. 50%, p = 0.02). There were no differences between the two groups in the frequency of PD-1 or PD-L1 expression. Mean TMB was 8.6 mutations/megabase and, using a TMB cut-off > 17 mutations/megabase to define high vs. low TMB, 6.7% of tumors were TMB-High. High TMB did not correlate with PD-1 (p = 0.50) or PD-L1 status (p = 0.52).

Conclusions: Molecular profiling differences between *TP53*-MT and *TP53*-WT SCCA indicate different carcinogenic pathways and biology, which may influence response to therapy. Low frequency mutations in several druggable genes may provide therapeutic opportunities for patients with SCCA.

Methods

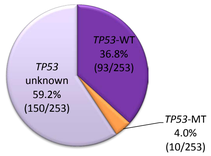
253 SCCA tumors were submitted for multiplatform testing using IHC, ISH, and/or NGS. 23 tumors underwent Illumina NextSeq (592 gene) testing. 107 tumors underwent NGS.

Chi-square tests were used to determine molecular differences between *TP53*-MT and *TP53*-WT tumors and for correlation of TMB to PD-1 and PD-L1 status.

Results

Median Age (Range)	59.2 (31 – 89)
Male	36% (91/253)
Female	64% (162/253)

Proportion of *TP53*-WT tumors



Specimen site of origin

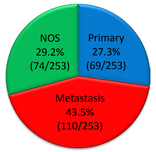
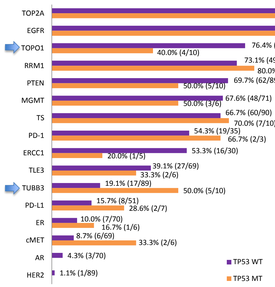


Figure 1. Demographics of the patient population. All *TP53*-MT tumors were metastases. NOS = not otherwise specified.

IHC profiles, *TP53*-WT vs. *TP53*-MT



NGS profiles, *TP53*-WT vs. *TP53*-MT

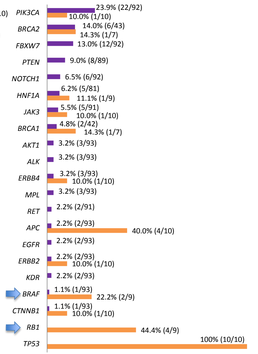
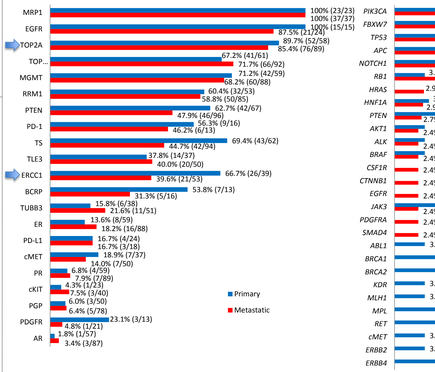


Figure 2. Comparison of *TP53*-WT (purple, N = 93) and *TP53*-MT (orange, N = 10) SCCA. Statistically significant differences were found in TOPO1 and TUBB3 expression. A comparison of NGS biomarkers revealed statistically significant differences in *BRAF* and *RB1* mutations (arrows indicate P < 0.05).

Results (continued)

IHC profiles, primary vs. metastatic



NGS profiles, primary vs. metastatic

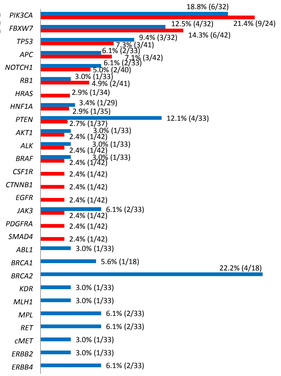


Figure 3. Comparison of primary (blue, N = 69) and metastatic (red, N = 110) SCCA. Primary and metastatic lesions were from unpaired samples. Statistically significant differences were found in ERCC1 and TOP2A expression (arrows indicate P < 0.05). A comparison of NGS biomarkers revealed no statistically significant differences.

Biomarker	Distribution
PD-1	55.8 (24/43)
PD-L1 (SP142)	26.5% (9/34)
Tumor mutational burden (TMB)	6.7% (2/23) with TMB above 17 Mean TMB 8.6 (Range 1 – 51)

Figure 4. Biomarkers conferring potential sensitivity to immune checkpoint inhibition (PD-1, PD-L1, and TMB).

Gene amplification by ISH

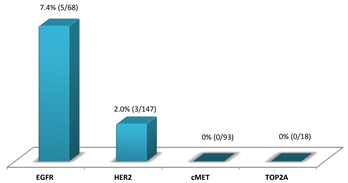


Figure 5. SCCA gene amplification by ISH (CISH and/or FISH). Higher percentages were detected in the EGFR/HER2-family of receptors, indicating a potential benefit to EGFR/HER2-directed therapy in a small, biomarker-selected population of patients.

Results (continued)

Mutation rates by 592 gene sequencing

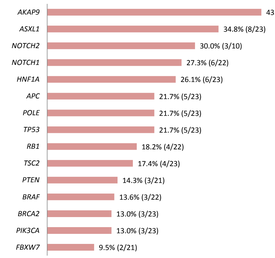


Figure 6. Selected common mutations observed in 23 SCCA tumors that underwent 592 gene testing.

Conclusions

- Molecular differences between *TP53*-MT and *TP53*-WT SCCA indicate different carcinogenic pathways and underlying tumor biology.
- The higher incidence of *BRAF* and *RB1* mutations in *TP53*-MT tumors compared to *TP53*-WT may reflects different oncogenic signaling pathways and a potential role for *BRAF* inhibition.
- The higher rate of ERCC1 expression in primary SCCA may indicate that platinum resistance is more common in primary tumors than metastatic tumors.
- PD-1 and PD-L1 expression were seen in both *TP53*-MT and *TP53*-WT tumors, suggesting *TP53* mutation status is not predictive of response to immune checkpoint inhibition.
- Frequent mutations in the *NOTCH* signaling pathway suggest a potential drug target in SCCA using *NOTCH* inhibitors.
- Low frequency of EGFR and HER2 gene amplifications suggest potential drug targets (e.g. trastuzumab) in select SCCA patients.

References

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3. Cacheux, W., A. Lievre, et al. (2016). "Mutational analysis of anal cancers demonstrates frequent PIK3CA mutations associated with poor outcome after salvage abdominoperineal resection". Br J Cancer. 114:1387-1394.