Molecular profiling of HPV positive and negative HNSCC

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Abstract

Background: Head and neck squamous cell carcinoma (HNSCC) is comprised of two subtypes: human papilloma virus (HPV)- and carcinogenic-associated (HPV-negative), both linked to inactivation of the TP53 pathway. We examined alterations in HPV positive (pos) and negative (neg) HNSCC to identify treatment options and elucidate differences in pathogenesis.

Methods: Eighty HNSCC cases (30 HPV-pos and 50 HPV-neg) were profiled using a multiplatform (IHC, NGS, ISH) approach (Caris Life Sciences, Phoenix, AZ) aimed at identification of biomarkers of therapeutic drug responses. To ensure comparison was between two distinct HNSCC etiologies, we filtered out TP53 wildtype HPV negative cases (n=20).

Results: HNSCC arising in the following anatomic sites were assessed: larynx, nasopharynx, oropharynx, oral cavity, tongue, and head and neck, NOS. HPV positivity was detected in: nasopharynx, oropharynx, tongue, NOS and head and neck, NOS. Statistically significant differences between HPV-pos and HPV-neg HNSCC were observed for ER(20% (6/30) vs. 3% (1/30); p=0.05), MGMT(27% (8/30) vs. 67% (20/30); p=0.004), RRM1 (43% (13/30) vs. 72% (21/29); p=0.035), and EGFR ISH (0% (0/15) vs. 27% (8/30); p=0.05). NGS detected variants in addition to TP53 in 50% of HPV-neg, including mutations in the following genes: APC, EGFR, PTEN, PDGFRA, GNAQ, HRAS, CDH1, IDH1, KDR and SMAD4. In HPV-pos cases NGS detected variants in 47% (14/30) including: APC, HNF1A, cMET, FBXW7, NRAS, PDGFRA, KDR, PIK3CA and PTEN. In patients with PTEN/PIK3CA mutations (both subgroups), 50%(4/8) exhibit loss of PTEN expression indicating benefit of mTOR inhibitors. Novel therapies based on NGS data include Wnt pathway inhibitors, multi-kinase inhibitors (imatinib), MEK inhibitors and alkylating agents based on mutations in APC, PDGFRA, GNAQ/NRAS and IDH1, respectively.

Conclusion: Common and HPV-specific biomarkers characterize HNSCC. ER over-expression indicates anti-hormonal therapies as a potential novel therapy option in HPV-pos, whereas alkylating agents may be of benefit in HPV-neg HNSCC. Novel therapies also include mTOR inhibitors based on alterations in PTEN/PIK3CA. Lower incidence of mutations in HPV-pos HNSCC indicates a need for a multi-platform approach in identifying theranostic biomarkers.

Background

There are two molecular subclasses of HNSCC: carcinogenic-associated and HPV-associated. Both inactivate the TP53 pathway (carcinogenic pathway is through somatic inactivating mutations of TP53, the viral pathway is through expression of viral oncoproteins which bind to and inhibit TP53). Loss of function of TP53 remains challenging to exploit therapeutically.

Overall, HPV-associated HNSCC have a more favorable prognosis and treatment response. However, a fraction of cases are recurrent and non-responsive to standard of care. Carcinogenic-associated HNSCC have limited treatment options due to being a TP53-driven cancer. Mutations in the p53 gene have been associated with decreased apoptosis and carcinogenic progression leading to decreased survival, poor response to therapy and higher risk of recurrence.

Current therapy approaches include surgical resection, chemo- and radiation therapy. The EGFR-targeted monoclonal antibody, cetuximab, is the only targeted therapy option for HNSCC, which has had limited efficacy in HPV +/- HNSCC. Molecular profiling using a multi-platform approach was utilized to elucidate unique potential therapeutic targets for HPV +/- HNSCC.

Methods

Eighty HNSCC cases referred to Caris Life Sciences between 2009 thru 2013 were evaluated. Specific testing was performed per physician request and included a combination of sequencing (next-generation sequencing [NGS]), protein expression (immunohistochemistry) and gene amplification (CISH or FISH). HPV status was determined by p16 IHC and/or HPV ISH (Ventana HPV high risk probes). 36/60 were determined by ISH only, 6/60 were determined by p16 IHC only and 18/60 were determined by ISH and p16. Positive cases were determined based on the interpretation guide from the manufacturers. HPV ISH and p16 IHC results were discordant in two cases, and ISH was used to determine the categorization for HPV status. To ensure comparison was between two distinct HNSCC etiologies, we filtered out TP53 wildtype HPV negative cases (n=20). Differentially expressed biomarkers were calculated by two-tailed Fisher Exact tests.

Results

Number of Patients	HPV +/TP53 WT (n=30)		HPV – / TP53 mutated (n=30)	
Average Age, years (range)	60.2 (48-78)		59.8 (27-87)	
Gender	Male: 27/30 (90%) Female: 3/30 (10%)		Male: 18/30 (60%) Female: 12/30 (40%)	
Male sex prevalence for HPV +	P= 0.0153			
Tumor Site	Oropharynx: 24 Nasopharynx: 1 Right parotid mass: 1 Head and neck, NOS: 3	Tongue, NOS: 1	Oropharynx: 14 Larynx: 3 Parotid gland: 2 Head and neck, NOS: 5	Mandible: 1 Nasopharynx: 1 Oral cavity: 2 Sinus: 2
Specimen Site Profiled	Primary: 11 Metastasis: 19		Primary: 14 Metastasis: 16	
Metastatic Sites	Brain: 1 Lung: 3 Liver: 2 Neck mass: 4	Lymph Nodes: 7 Chest wall: 1 Skin: 1	Bone: 3 Lung: 3 Liver: 1 Neck Mass: 5	Lymph Nodes: 2 Pharynx: 1 Pleural Effusion: 1

Table 1– Patient Characteristics – samples profiled represent the full range of the disease as evidenced by 25 localized and 35 metastatic HNSCC. Majority of specimens profiled are from treatment-refractory patients.

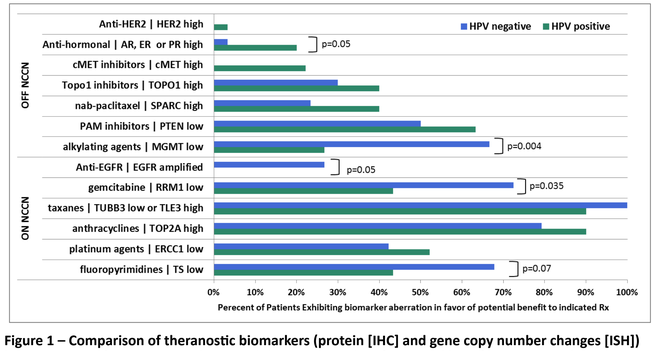

Figure 1 – Comparison of theranostic biomarkers (protein [IHC] and gene copy number changes [ISH]) between HPV +/- HNSCC. Anti-hormonal therapies may be of potential benefit in HPV + with 20% overexpressing AR, ER or PR, compared to 3% in HPV – . Higher frequency of low MGMT and RRM1 expression in HPV – is suggestive of temozolomide or gemcitabine benefit (67% vs. 27% and 72% vs. 43%, respectively). EGFR amplification was observed in 27% of HPV - vs. 0% in HPV +, which is suggestive of anti-EGFR therapy, and a trend was observed for high frequency of low expression of TS in HPV – vs. HPV + (68% vs. 43%), which associates with benefit to fluoropyrimidines. Several "off-compendium" agents were identified as having potential benefit based on expression rates (low or high) of various theranostic markers, for both HPV subgroups of HNSCC.

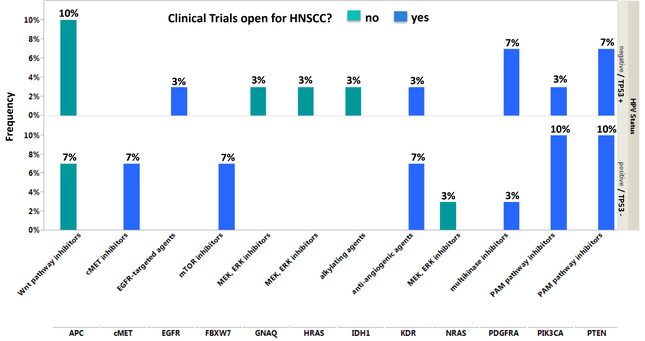

Figure 2 – Targetable aberrations identified by NGS and associated therapies. 47% (14/30) of HPV-associated HNSCC have mutations identified by NGS, all of which are considered targetable with drugs in clinical trials, or FDA-approved for other tumor types, with the exception of a mutation in HNF1A. 37% (11/30) of HPV-negative HNSCC have mutations identified by NGS, in addition to TP53, all of which are considered targetable, with the exception of mutations in SMAD4 and CDH1. **All agents identified by NGS aberrations are off NCCN compendium and not approved for use in HNSCC.** Agents that are being studied for use in HNSCC and available through clinical trials are indicated by the blue bars. Agents not available in clinical trials, designed specifically for HNSCC, are indicated by the green bars.

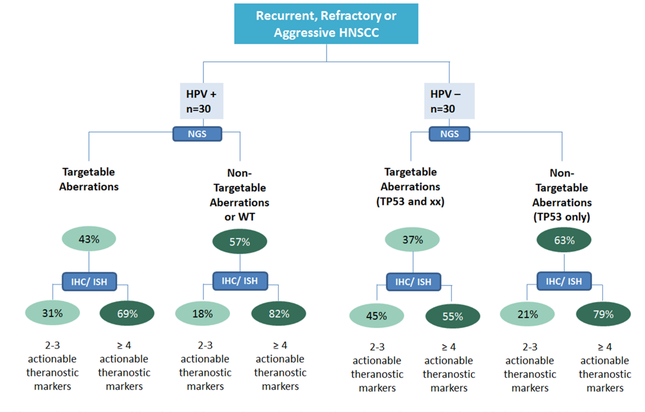

Figure 3 – CMI profiling identifies actionable alterations by IHC and ISH in 100% of HNSCC which lack actionable mutations by NGS. Over half of HNSCC (both HPV +/-) lack targetable mutations. Applying the CMI multiplatform approach, IHC and ISH platforms identified actionable alterations in 100% of cases, with 82% and 79% having ≥ 4 actionable markers, in HPV +/- patients, respectively.

	PIK3CA pathway alterations				Patient Characteristics		
Patient	Gene	Mutation	Exon/Domain	PTEN IHC	Primary Tumor	Specimen Profiled	HPV status
Pt. 1	PTEN	T319fs	Exon 8	0+ 100% (loss)	tonsil	brain metastasis	positive
	PIK3CA	E542K	Exon 9				
Pt. 2	PIK3CA	E545K	Exon 9	1+ 80% (intact)	base of tongue	base of tongue	positive
	PIK3CA	H1048R	Exon 20				
Pt. 3	PIK3CA	E545K	Exon 9	1+ 40% (loss)	tongue, nos	right neck mass metastasis	positive
Pt. 4	PTEN	Q17fs	Exon 1	1 + 5% (loss)	tonsil	left neck mass metastasis	positive
Pt. 5	PTEN	Q17E	Exon 1	1+ 90% (intact)	base of tongue	base of tongue	positive
Pt. 6	PTEN	Y336X	Exon 8	1+ 2% (loss)	floor of mouth	mandible metastasis	negative
Pt. 7	PTEN	R335X	Exon 8	1+ 70% (intact)	parotid gland	lung metastasis	negative
Pt. 8	PIK3CA	H1074R	Exon 20	1+ 85% (intact)	parotid gland	lymph node metastasis	negative

Table 2. PIK3CA and PTEN are the most frequently activated (17%) targetable mutations in both subgroups of HNSCC. All mutations have previously been reported in the literature. Inhibitors of the PIK3CA pathway are currently being developed and therefore are ideal candidate agents for use in HNSCC, which lacks targeted therapy options (cetuximab is the only FDA-approved targeted therapy used for HNSCC). Fifty percent of patients with PTEN/PIK3CA mutations harbor loss of PTEN expression by IHC, a resistance mechanism recently identified in a metastatic breast cancer patient treated with a PIK3CA inhibitor, BYL719 (*Castel, et al. AACR 2014*).

Conclusions

- 60 HNSCC samples, including localized and metastatic disease from various tumor sites, were profiled with a multi-platform approach using immunohistochemistry, *in situ* hybridization and next-generation sequencing.
- IHC and ISH platforms identified several therapy options in both subgroups of HNSCC, including agents on and off NCCN compendium for HNSCC. Differentially expressed theranostic markers included hormone receptors for HPV + and MGMT, RRM1, TS and EGFR for HPV -/TP53+
- NGS platform identifies targetable mutations in 47% of HPV + and 37% of HPV-/TP53+ HNSCC. The most commonly mutated pathways across subgroups include PIK3CA/PTEN and Wnt signaling pathways. Inhibitors of the PIK3CA pathway are in clinical development, and recent data suggests a potential role for PTEN loss as detected by IHC as a resistance mechanism. Half of patients with PIK3CA/PTEN mutations harbor PTEN loss in our cohort.
- CMI profiling which includes multiple platforms provides therapy options based on IHC and ISH in 100% of HNSCC which did not have targetable mutations by NGS.
- The Caris analysis includes measurement of the major, clinically actionable molecular changes in HNSCC. HPV pos and neg HNSCC, have some unique therapy options as identified by IHC, ISH and NGS. The PIK3CA/PTEN pathway is the most promising druggable target in HNSCC, and profiling with several platforms will prove useful for determining de novo and acquired resistance mechanisms.

References

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2. Stransky, N., J.R. Grandis, et al. (2011). "The Mutational Landscape of Head and Neck Squamous Cell Carcinoma." Science 333(6046):1157-1160