Differences in Biomarker Expression in HNSCC According to p53 Status

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Abstract

Background: Patients with p53 wildtype head and neck squamous cell carcinoma (HNSCC) tend to be HPV-positive, which associates with better prognosis. The purpose of this study was to explore biomarker expression profiles for insight into molecular differences in HNSCC patients based on p53 status.

Methods: P53 gene sequencing using the AmpliChip p53 microarray (Roche Molecular Systems, Inc.) was attempted on 61 HNSCC patients previously tested with Caris Target Now TM tumor profiling service. DNA was extracted from a FFPE sample, amplified and processed on the AmpliChip p53 microarray to detect single base pair substitution and deletion mutations in exons 2 - 11 and their flanking splice sites in the TP53 gene (GenBank X54156). EGFR FISH , HER2 IHC and 22 other predictive biomarkers, e.g. TS, TOPO2A, MGMT, etc., were assayed and retrospectively analyzed. All tests were performed in a CLIA-certified lab and interpreted by board-certified pathologists or cytogeneticists. Statistical analysis was performed using SPSS.

Results: 52 cases provided sufficient quality DNA for p53 analysis and results revealed a mutation rate of 25% in HNSCC patients. Interestingly, only EGFR FISH and HER2 IHC (p=.002 and p=.004, respectively) were differentially expressed in wildtype vs. mutated p53. Matched-pair analysis in the p53 mutated subgroup (n=13) showed no significant trend regarding EGFR status (p=.763) but a slight trend towards HER-2 negativity (p=.020). In the p53 wildtype subgroup (n=39), a strong association with EGFR FISH non-amplification (n=28, 71.8%, p<.001) as well as HER-2 negativity (n=38, 97.4%, p<.001) was shown.

Conclusion: To our knowledge, this is the first analysis of differential biomarker expression profiles in HNSCC based on p53 status. We hypothesize that the absence of EGFR amplification in the p53 wildtype cancers may be a contributing factor to the improved prognosis observed in HPV-positive HNSCC. Additionally, the strong association between p53 wildtype HNSCC patients and EGFR non-amplification suggests EGFR-targeted therapies like cetuximab would likely fail in p53 wildtype patients.

References

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Background

Head and neck cancer is the sixth most common cancer worldwide, with ninety percent of head and neck cancers being diagnosed as squamous cell carcinomas (HNSCC). Despite advances in molecular medicine, HNSCC remains an aggressive disease with poor prognosis and high risk of recurrence.

Tobacco use and alcohol consumption have long been the top-ranked risk factors. In addition, the human papillomavirus (HPV) has been identified as a causative agent in a growing subset of HNSCC, and now defines two molecular subclasses of HNSCC, HPV-positive (viral etiology) and HPV-negative (carcinogenic etiology). The tumor suppressor, p53 is at the core of both molecular subclasses, of which mutation status inversely correlates with HPV status. Overall, HPV-positive (p53 wildtype) HNSCC patients tend to have a more favorable prognosis and better overall response to standard therapy.

The aim of this study was to examine the biological basis of this difference in prognosis and response. Due to controversies surrounding HPV testing, we chose the AmpliChip p53 (Roche Molecular Systems, Inc.) assay to delineate our cohort into p53 wildtype and p53 mutated subgroups. Using various molecular testing methods, including immunohistochemistry, fluorescent in situ hybridization and sequencing, we obtained biomarker expression profiles for p53 wildtype and mutated HNSCC to identify a potential explanation for differences in prognosis.

Methods

TP53 gene sequencing using the AmpliChip p53 microarray (Roche Molecular Systems, Inc.) was attempted on 61 HNSCC patients previously tested with Caris Target Now TM tumor profiling service. DNA was extracted from FFPE samples, amplified and processed on the AmpliChip p53 microarray to detect single base pair substitution and deletion mutations in exons 2 - 11 and their flanking splice sites in the TP53 gene (GenBank X54156). The expression of AR (AR441), BCRP (6D171), cKIT (polyclonal), cMYC (8F11), COX-2 (SP21), EGFR (2-18C9), ER (SP1), p53 (DO-7), PDGFR (polyclonal), Her2 (4B5), ERCC1 (8F1), MGMT (MT23.3), MRP1 (33A6), PGP (C494), PR (1E2), PTEN (6H2.1), RRM1 (polyclonal), SPARC monoclonal (122511), SPARC (polyclonal), TOPO1 (1D6), TOPO2A (3F6) and TS (TS106) were assayed on a Ventana or Dako platform in a CLIA-certified lab and evaluated independently by board-certified pathologists. Results were categorized by defined cutoff points based on published evidence into above threshold, below threshold (intermediate) or negative. Reflexive testing for EGFR and HER2 fluorescent in situ hybridization (FISH) based on PTEN IHC and HER2 IHC results, respectively, was also performed. EGFR FISH positivity was defined as increase in gene copy number (>40% of cells displaying >4 copies of EGFR signal) or gene amplification (gene/chromosome per cell ratio of ≥ 2, or ≥ 15 copies of the genes per cell in ≥ 10% of analyzed cells). HER2 FISH amplification was defined as HER2/neu:CEP 17 signal ratio of > 2.2. Additional tests requested by the ordering physicians included TOPO2A and ALK FISH, and direct sequence analysis of BRAF, cKIT, EGFR, KRAS and PIK3CA which were sequenced in exons 11 and 15; exons 9, 11, 13 and 17; exons 18-21; exons 12,13 and 61, and exons 9 and 20, respectively. Statistical analysis was performed using SPSS (PASW statistics 17) for both parametric and non-parametric (Chi Square, Mann-Whitney) tests of independence.

Results

52 cases provided sufficient quality DNA for p53 analysis and results revealed a mutation rate of 25% in HNSCC patients. Matched-pair analysis in the p53 mutated subgroup (n=13) showed no significant trend regarding EGFR status (p=.763), however half of the mutated patients with EGFR FISH data were EGFR FISH positive (see Figure 1b.). In the p53 wildtype subgroup (n=39), a strong association with EGFR FISH non-amplification (n=28, 71.8%, p<.001) as well as HER-2 negativity (n=38, 97.4%, p<.001) was shown. No other statistically significant associations were found.

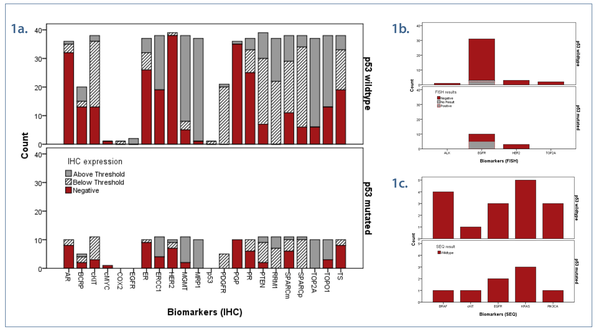

Figure 1a-c – Differential expression by biomarker and assay, IHC **(a)**, FISH **(b)**, SEQ **(c)**, stratified by p53 mutation status (WT=39, mutated=13). Y axis denotes subsample size (n).

Based on these data, the p53 wildtype group may represent a more genetically stable subgroup. We further examined the data by classifying the p53 mutated cases into disruptive and non-disruptive mutations based on a previously published HNSCC study [2]. Data from three patients was excluded due to the absence of EGFR data and identification of a splice variant occurring in intron 5 which has unknown functional impact. After delineating the mutated patients, we found that 40% of patients with disruptive p53 mutations were EGFR FISH positive vs. 30% EGFR FISH negative. In contrast, 10% of patients with nondisruptive p53 mutations, were EGFR FISH positive and 20% EGFR negative. Although interpretation of these results must be taken with caution, these data may help explain recent treatment outcome reports of the SPECTRUM trial, where only HPV-negative (likely p53 mutated) patients experienced an overall survival benefit from the EGFR-targeted therapy, panitumumab [3]. Due to presence of p53 mutations and inherent genetic instability, this subgroup of patients will likely be more resistant to standard chemo- and radiation therapy, thus targeted agents like the EGFR-targeted monoclonal antibodies may be ideal.

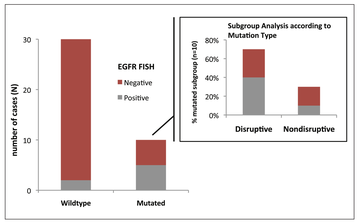

Figure 2 – EGFR FISH by p53 mutation status (left) and subgroup analysis of p53 mutated stratified by disruptive and nondisruptive (inlay). Y axis denotes subsample size (n) (left) or percent distribution in mutated cohort (inlay).

Study Highlights

- Amplichip p53 assay (Roche Molecular Systems, Inc.) was used to delineate the two molecular subclasses of HNSCC, yielding a mutation rate of ~25%
- Biomarker expression profiles were compared between p53 wildtype and mutated patients. EGFR FISH non-amplification and HER2 IHC negativity were the only statistically significant biomarkers differentially expressed in p53 wildtype and mutated subgroups.
- Further analysis in the p53 mutated subgroup revealed a slight tendency towards EGFR FISH positivity in HNSCC patients with disruptive p53 mutations.
- The p53 wildtype cohort may exhibit a more genetically stable profile and subsequently a better prognosis, as evidenced by HER family down-regulation.

Conclusions

- Amplichip p53 assay was used to delineate our HNSCC cohort into the two main subclasses, p53 wildtype (likely HPV positive) and p53 mutated (likely HPV negative).
- Biomarker expression profiles yielded statistically significant associations for p53 wildtype subgroup with EGFR FISH non-amplification and HER2 IHC negativity.
- Classification of p53 mutations revealed a slight tendency for p53 mutated (disruptive) patients to be EGFR FISH positive.
- HER family deregulation in p53 mutated patients may reflect a genetically instable profile leading to an overall poorer prognosis and response to therapy.
- Regarding therapy options, EGFR-targeted therapies like cetuximab may not be a suitable choice for the p53 wildtype subgroup. However, EGFR IHC should be considered before ruling out EGFR-targeted treatment in this subgroup.
- In contrast, the p53 wildtype/EGFR non-amplified subgroup may more likely benefit from standard chemo- (fluorouracil, platinum agents) and radiation therapies. Incorporation of the remaining biomarker results may identify treatment options on an individual basis.