Differences in Biomarker Expression in HNSCC According to p53 Status

Rebecca Feldman, Ph.D., Ariane Kemkes, Ph.D., Joanne Xu, Ph.D., Richard Blevins, Ph.D., Paula Esmay, M.S., Leslie Battaglia, C.G.(ASCMP), C.M., Katrina Radebach, C.G.(ASCP), C.M., Inga Rose, B.S., Curtis Johnston, M.D., Zoran Gatalica, M.D., DSc.

Caris Life Sciences Phoenix, AZ, Dallas, TX

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 HPV-negative p53 patients, based on p53 status. Methods: p53 gene sequencing using the AmpChip p53 microarray (Roche Molecular Systems, Inc.) was attempted on 61 HNSCC patients previously tested with Caris Target Now HPV tumor profiling service. DNA was extracted from FFPE samples, amplified, and processed on the AmpChip p53 microarray to detect single base pair substitution and deletion mutations in exons 2-11 and their flanking splice sites in the p53 gene (GenBank #NG_009556). EGFR FISH, HER2 IHC, and other cytogenetic biomarkers, e.g., T5P02A, T5P01A, MGMT, etc., were assessed and retrospectively analyzed. All tests were performed in a CLIA-verified and interpreted by board-certified pathologists or cytopathologists. 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 p53 wildtype patients. Interestingly, only HER2 FISH and HER2 IHC (one per 500, respectively) were differentially expressed in wildtype vs. mutated p53. Matched-pair analysis in the p53 mutated subgroup (n=11) showed no significant trend regarding EGFR FISH (p=0.763) but a slight trend towards HER2 FISH positivity (p=0.021). In the p53 wildtype subgroup (n=39), strong association with EGFR FISH non-amplification (≥80%), HER2 FISH non-amplification, and HER2 IHC negativity was observed. Overall, HPV-positive (p53 wildtype) HNSCC patients have tendency for a more favorable prognosis and better overall response to therapy.

Conclusions: 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 cancer may be a contributing factor to the improved survival observed in HPV-negative HNSCC. Additionally, the strong association between p53 wildtype HPV-negative patients and EGFR non-amplified suggests EGFR-targeted therapies like cetuximab would likely fail in p53 wildtype HNSCC. Additionally, the strong association between p53 wildtype HNSCC patients and EGFR non-wildtype cancers may be a contributing factor to the improved prognosis observed in HPV-positive patients. 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 AmpChip p53 (Roche Molecular Systems, Inc.) assay to delineate our cohort into p53 wildtype and p53 wildtype subgroups. Using various molecular testing methods, including immunohistochemistry, fluorescence in situ hybridization and sequencing, we obtained biomarker expression profiles for p53 wildtype and mutated HNSCC to identify a potential explanation for differences in progression.

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 carcinoma (HNSCC). Despite advances in molecular medicine, HNSCC remains an aggressive disease with poor prognosis and high risk of recurrence. Tobacco and alcohol consumption have lengthened 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 subtypes of HNSCC: HPV-positive (vacant sites) and HPV-negative (background sites). The tumor suppressor p53 in the case of both molecular subtypes 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 therapy. To our knowledge, this is the first analysis of differential biomarker expression profiles in HNSCC according to p53 status. We hypothesize that the absence of EGFR amplification in the p53 wildtype cancer may be a contributing factor to the improved survival observed in HPV-negative HNSCC. Additionally, the strong association between p53 wildtype HPV-negative patients and EGFR non-amplified suggests EGFR-targeted therapies like cetuximab would likely fail in p53 wildtype HNSCC. Additionally, the strong association between p53 wildtype HNSCC patients and EGFR non-wildtype cancers may be a contributing factor to the improved prognosis observed in HPV-positive patients.

Methods

p53 gene sequencing using the AmpChip p53 microarray (Roche Molecular Systems, Inc.) was attempted on 61 HNSCC patients previously tested with Caris Target Now HPV tumor profiling service. DNA was extracted from FFPE samples, amplified and processed on the AmpChip p53 microarray to detect single base pair substitution and deletion mutations in exons 2-11 and their flanking splice sites in the p53 gene (GenBank #NG_009556). The expression of 148 ARs (2244) were analyzed by IHC in different tumor subsets or subgroups. EGFR FISH was determined using the AmpliChip p53 assay (Roche Molecular Systems, Inc.) to delineate the two molecular subgroups of HNSCC. In addition, the study aimed to identify more genomically stable and potentially more resistant to standard chemotherapy and radiation therapy; thus targeted agents like the EGFR monoclonal antibodies may be ideal.

Study Highlights

• HPV p53 assay (Roche Molecular Systems, Inc.) was used to delineate the two molecular subgroups 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 p53 wildtype in patients with HPV-negative status.
• The p53 wildtype cohort may exhibit a more genetically stable profile and subsequently a better prognosis, as evidenced by HER2 family down-regulation.

Conclusions

• HPV p53 assay was used to delineate our HNSCC cohort into the two main subgroups, 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 mutations) to be HPV-negative.
• HER2 family downregulation in p53 mutated patients may reflect a genetically unstable profile leading to an overall poorer prognosis and response to therapy.
• Regarding therapy options, EGFR Farnesyltransferase inhibitors like cetuximab may not be a suitable choice for the p53 wildtype subgroup. However, EGFR IHC should be considered before ruling out EGFR-targeted therapy in this subgroup.
• In contrast, in p53 mutated patients, EGFR IHC or HER2 FISH non-amplified subgroups may offer some benefit from standard chemotherapy (fluorouracil, platinum agents) and radiation therapy. Incorporation of the remaining biomarker results may identify treatment options on an individual basis.

References


Figure 1 – Differential expression by biomarker and assay. (A) (B), (C) (D) (E) (F) (G) (H) (I) (J) (K) (L) (M) (N) (O) (P) (Q) (R) (S) (T) (U) (V) (W) (X) (Y) (Z) (AA) (BB) (CC) (DD) (EE) (FF) (GG) (HH) (II) (JJ) (KK) (LL) (MM) (NN) (OO) (PP) (QQ) (RR) (SS) (TT) (UU) (VV) (WW) (XX) (YY) (ZZ).

Figure 2 – Overview of 52 cases with sufficient quality DNA for p53 analysis and results revealed a mutation rate of 25% in p53 wildtype patients. Interestingly, only HER2 FISH and HER2 IHC (one per 500, respectively) were differentially expressed in wildtype vs. mutated p53. Matched-pair analysis in the p53 mutated subgroup (n=11) showed no significant trend regarding EGFR FISH (p=0.763) but a slight trend towards HER2 FISH positivity (p=0.021). In the p53 wildtype subgroup (n=39), strong association with EGFR FISH non-amplification (≥80%), HER2 FISH non-amplification, and HER2 IHC negativity was observed. Overall, HPV-positive (p53 wildtype) HNSCC patients have a more favorable prognosis and better overall response to therapy.