Molecular profile of head and neck squamous cell carcinomas bearing p16 high phenotype

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Received 20 May 2012; revised 13 November 2012 & 16 December 2012; accepted 3 January 2013

Background: We sought to determine biomarker expression differences in head and neck squamous cell cancers (HNSCCs) based on p16/human papillomavirus (HPV) classification. In addition, our aim was to explore how expression of biomarkers is modulated after E6/E7 repression in HPV16+ oropharyngeal cancer cells.

Methods: HPV16+ and HPV− HNSCC cells were infected with retroviruses expressing short hairpin RNA targeting HPV16 E6/E7. Components of the epidermal growth factor receptor (EGFR) pathway before and after E6/E7 gene silencing were analyzed by immunoblotting and qRT–PCR. Protein expression of 13 biomarkers was analyzed using AQUA on a tissue microarray (TMA). The HPV16 status was determined using HPV16 in situ hybridization (ISH).

Results: In HPV16+ cells, E6/E7 silencing was associated with PTEN upregulation and reduction of phosphorylated EGFR. Tumors were classified into four categories based on the HPV and p16 status. HPV+/p16+ tumors expressed significantly higher levels of E-cadherin (P = 0.003), PTEN (P = 0.004), lower levels of PI3Kp110 and β-catenin (P = 0.07). There was a significant difference in overall survival (OS, P = 0.016) among the four subsets. The median OS was 24.83 months for p16−/HPV− patients, 11.63 for p16−/HPV+ patients and was not reached for p16+/HPV− and p16+/HPV+ groups.

Conclusions: Aberrant EGFR signaling contributes to malignant conversion of HPV16+ HNSCC cells. These results validate β-catenin as a distinct biomarker in HPV+/p16+ HNSCC. Wnt signaling inhibitors merit exploration in HPV+/p16+ HNSCC.

Key words: EGFR, HNSCC, p16+, Wnt

introduction

Worldwide, an estimated 644 000 new cases of head and neck squamous cell cancers (HNSCCs) are diagnosed per year [1]. Tobacco abuse and alcohol consumption account for majority of cases of HNSCC. Growing evidence over the past two decades suggests that human papillomavirus (HPV) 16 infection is causally associated with subset of oropharyngeal squamous cell cancers in individuals who have little or no history of alcohol or tobacco use [2, 3].

These HPV-associated HNSCCs represent a distinct molecular and clinical entity. Tobacco-associated oropharyngeal squamous cell carcinoma (OSCC) is characterized by downregulation of tumor-suppressor p16 protein and frequently by mutation of p53 gene. To the contrary, in HPV-associated OSCC, abrogation of p53 and Rb tumor-suppressor pathways occurs via binding and degradation of p53 and Rb proteins by E6 and E7 viral oncoproteins, respectively [4]. P53 and pRb pathways are active but dormant in HPV16+ OSCC cells. Retrovirus-mediated repression of E6 and E7 viral oncogene expression induced apoptosis and restoration of expression of p53 and Rb proteins in HPV16+ oropharyngeal cancer cell lines [5].

HPV-associated OSCC have superior outcomes in terms of overall and disease-free survival compared with non-HPV-associated counterparts. Most published studies strongly support the notion that HPV-associated OSCC represents a new disease entity distinct from tobacco-associated OSCC for which novel therapeutic strategies are needed. An important goal of new approaches for favorable prognosis HPV-associated cancers will be minimization of devastating side-
effects of intensified treatment developed for poor prognostic subsets. HPV-targeted therapies such as therapeutic vaccines seem to be a promising therapeutic strategy against HPV-associated OSCC. Data derived from cervical cancer, however, regarding the efficacy of therapeutic vaccines are to date discouraging. The identification of biomarkers that distinguish HPV-associated from non-HPV OSCC will shed light on pathogenesis of, and possible paths to targeted therapies against, these viral-induced tumors.

In our previous study, we analyzed paraffin-embedded OSCC specimens from 77 patients classified into a three-class model [HPV+, HPV inactive (HPV16+/-p16+) and HPV active (HPV16+/p16+)]. These samples were queried for expression of 14 tumor progression proteins using AQUA. Protein expression between groups was assessed by analysis of variance. There were significant differences in expression of β-catenin (P = 0.009), epidermal growth factor receptor (P = 0.009) and vascular endothelial growth factor (P = 0.028) between groups [6]. In the present study, we sought to validate these results in an independent cohort of HNSCC patients. In addition, we aimed to validate the prognostic value of p16 in HNSCC. Furthermore, we aimed to determine whether the expression of key components of the epidermal growth factor receptor (EGFR) signaling network is regulated by E6/E7 viral oncoproteins. The conceptual correlation of these two parallel projects lies on the exploration of strategies to treat these two molecular subsets of HNSCCs.

patients and methods

The inclusion criteria were histologically confirmed primary HNSCC treated at Yale-New Haven Hospital and Aristotle University Hospital between 1992 and 2005, with either external beam radiotherapy or gross total surgical resection and postoperative radiotherapy (n = 102). The exclusion criteria included presentation with metastatic or recurrent disease or failure to receive a full course of radiation therapy, Patients with incomplete clinical-pathological data or those lost to follow-up were also excluded.

tissue microarray construction

Following institutional review board approval, tissue microarray (TMA) was constructed as previously described [7]. Tissue cores 0.6 mm in size were obtained from paraffin-embedded formalin-fixed tissue blocks from the archives of the Yale University and Aristotle University of Thessaloniki Department of Pathology. Further details are described in the supplementary Materials and Methods, available at Annals of Oncology online.

quantitative immunohistochemistry and automated image acquisition and analysis

TMAs were deparaffinized and stained as previously described [8]. Tumors were evaluated for expression of 14 biomarkers (p16, E-cadherin, β-catenin, EGFR, MET, STAT3, ERK1/2, pAKT, pI3Kp85, pI3Kp110, PTEN, p53, Bcl2, NFkB, supplementary Table S2, available at Annals of Oncology online). Methodology and scoring details are provided in the supplementary Materials, available at Annals of Oncology online.

statistical analysis

Details are provided in the supplementary Materials, available at Annals of Oncology online.

cell lines and retrovirus-mediated shRNA silencing

Cell line 147T (93VU147T) was a kind gift of Dr Renske Steenbergen and the 090 cell line (UPCI:SCC090) was a kind gift of Dr Susanne Golin. 147T and 090 oropharyngeal cancer cell lines stably expresses the HPV16 E6/E7 transcript. The methodology of retrovirus-mediated shRNA silencing is provided in the supplementary Materials, available at Annals of Oncology online.

western blotting and quantitative real-time reverse transcription-PCR

Methodology is provided in the supplementary Materials, available at Annals of Oncology online.

high-risk HPV ISH

The details of high-risk HPV in situ hybridization (ISH) are provided in supplementary Materials, available at Annals of Oncology online.

results

clinical and pathological variables

Our study included 102 patients with histologically confirmed HNSCC (primary, recurrent). The median follow-up was 23.1 months (Demographic and clinicopathological variables for the cohort are summarized in supplementary Table S1, available at Annals of Oncology online). Tumors with high versus low p16 expression levels did not differ by patient gender, tumor TNM stage or tumor grade in this moderately sized sample. However, oropharyngeal tumors had higher expression levels of p16. All HPV+/p16+ tumors were oropharyngeal.

quantitative immunohistochemistry for p16 protein expression

As visualized by fluorescent immunohistochemistry, p16 displayed mainly cytoplasmic staining (Figure 1A –C). Normalized AQUA scores for p16 expression in tumors ranged from 0.77 to 81.32. Tumor p16 expression followed a skewed distribution as expected for a cancer tissue biomarker (Figure 1D). To assess for intratumor heterogeneity of p16 expression and control for reproducibility of the assay, we compared AQUA scores from redundant tumor cores and observed significant correlation (R = 0.85). AQUA scores were averaged between the two histo spots and final scores ranging from 1.73 to 61.62 were obtained for 102 patients. AQUA scores were split at the median (AQUA score 3.27) to yield binomial (high versus low) variables. Patients with tumor p16 expression <3.27 were classified as low expressors (n = 51), and patients with tumor p16 expression >3.27 were classified as high expressors (n = 51) [8].

in situ hybridization for high-risk HPV detection

Analysis of AQUA data showed a surprisingly high p16 positivity rate (40%) in laryngeal cancer specimens. In order to test whether this high rate is associated with high-risk HPV infection, laryngeal cancer specimens were tested for high-risk HPV infection by ISH using a set of probes for high-risk HPV detection. Specifically, of 19 p16- laryngeal cancer specimens included in our study, 10 (53%) had sufficient tissue for HPV
detection by ISH; of those, two were HPV+ and eight were HPV−.

univariate survival analysis

progression-free survival

The status of p16 expression was evaluated for association with progression-free survival (PFS) using the log-rank statistic to determine significance. A non-significant trend was observed between high p16 expression and superior PFS; patients with high p16 had a higher PFS at 5 years compared with patients with low p16. The cumulative PFS at 5 years was 62.6% for high p16 expressers versus 39.4% for low expressers (supplementary Table S3, available at Annals of Oncology online). The median PFS has not been reached and the mean PFS is 42.7 months for high expressers, compared with a median of 26.6 months and a mean of 33.8 months for those with low tumor p16 expression by AQUA (Figure 2A). This difference in PFS trended towards significance (P = 0.07; log-rank test). Analysis of PFS according to the p16/HPV status revealed significant difference (P = 0.001; Figure 2C).

overall survival

The expression status of p16 was also evaluated for association with OS. Kaplan–Meier analysis demonstrated that there was a significant correlation between high p16 expression and improved OS. Patients with high p16 levels had significantly better OS (median not reached, mean 45.5 months), whereas patients with low p16 levels had a shorter OS (median 27.6 months, mean 35.7 months) (P = 0.025; log-rank test). Patients with high p16 expression had an OS at 5 years of 62.1% compared with 41.3% for patients with low p16 (Figure 2B). Overall survival (OS) according to the p16/HPV status was determined (Figure 2D). There was a significant difference in OS (P = 0.016) among the four subsets. The median OS was 24.83 months for the p16−/HPV− patients, 11.63 for the p16−/HPV+ patients and was not reached for the p16+/HPV− and p16+/HPV+ groups. At 5 years, 83.3% of the p16+/HPV+ patients, 70.3% of the p16+/HPV− and 40.2% of the p16−/HPV− patients were alive, whereas none of the p16−/HPV+ patients survived.

multivariable survival analysis

Using the Cox proportional hazards model, we carried out multivariable analysis to assess the prognostic value of p16 expression by AQUA for OS. We included the following prognostic variables in the regression model: gender, TNM stage, tumor grade and tumor site. A 72% decrease in the risk of death was estimated for patients with high p16 protein expression (HR = 0.277, 95% CI 0.10–0.77, P = 0.014; supplementary Table S4, available at Annals of Oncology online).

Figure 1. Fluorescent immunohistochemistry for automated analysis. (A) Cytokeratin was used to identify tumors. (B) Pseudocolored co-localization image demonstrating compartment assignment. Cytokeratin-Cy3 (green) was used to define a non-nuclear compartment; DAPI (blue) was used to define a nuclear compartment. (C) Cy5 (red) was used to identify p16. Original magnification ×20. (D)AQUA analysis showed a left skewed distribution for p16 tumor expression.
When the p16/HPV categories were included in the multivariate model, no prognostic significance was demonstrated for any of the variables (data not shown).

**AQUA quantitative analysis of protein expression**

Using AQUA quantitative fluorescent immunohistochemistry, protein expression levels for 13 target proteins were determined and compared by p16 status. *Post-hoc* comparisons demonstrated that p16+ tumors had significantly higher β-catenin (*P* = 0.006), E-cadherin (*P* = 0.015), bcl-2 (*P* = 0.005), NFκB (*P* = 0.007), PI3Kp110 (*P* = 0.035) and PTEN (*P* = 0.0009) expression levels. There was no significant association between p16 status and the rest of the biomarkers (EGFR, MET, STAT3, ERK1/2, pAKT, PI3Kp85 and p53). The results of fluorescent immunohistochemistry and AQUA analysis are summarized in Table 1. The biomarkers were analyzed by HPV/p16 category and the results are listed in Table 2. HPV+/p16+ tumors expressed significantly higher levels of E-cadherin (*P* = 0.003), PTEN (*P* = 0.004), β-catenin (*P* = 0.07) and bcl-2 (*P* = 0.08), as well as lower levels of PI3Kp110 (*P* = 0.022).

**EGFR/PTEN/AKT signaling in relation to e6/e7 oncogene expression**

The protein levels of EGFR, phosphorylated EGFR (pEGFR), AKT, phosphorylated AKT (pAKT), PTEN and phosphorylated PTEN (pPTEN) were analyzed before and after E6/E7 gene silencing in 147T and 090 HPV16+ oropharyngeal cancer cell lines by western blotting. E6/E7 repression was achieved by the retrovirus-mediated expression of a shRNA oligo (shRNAH4) that has been previously shown to downregulate the expression of E6 and E7 viral...
oncogenes [5]. Quantitative RT-PCR analysis of E6 and E7 expression and western blot analysis of p53 and pRb protein levels were carried out in order to confirm the E6/E7 repression in retrovirus-infected (RV-shRNAHN4) cells as previously described [5]. The protein expression analysis for EGFR, AKT and PTEN showed that only the protein levels of PTEN were significantly upregulated after E6/E7 gene silencing (Figure 3A). To study whether the nuclear protein levels of PTEN in RVshRNAHN4-infected 147T and 090 cells are also upregulated, nuclear fractions of RVshRNAHN4 and RVControl shRNA-infected cells were subjected to immunoblotting with antibodies recognizing PTEN, β-actin, histone H3 and GRP78/Bip, 48 h after retrovirus infection. Actin levels were evaluated to determine equivalent loading, whereas the detection of histone H3, a nuclear protein, and GRP78/Bip, an endoplasmatic reticulum protein, was used to indicate nuclear purity. As shown in Figure 3B, nuclear levels of PTEN were significantly upregulated after E6/E7 repression. To investigate a possible activation of the AKT signaling pathway in HPV+ cell lines as a result of PTEN protein downregulation, we analyzed the protein levels of unphosphorylated and phosphorylated AKT (Ser473) 48 h after retrovirus infection. Western blot analysis showed that all the Akt proteins were in the unphosphorylated state in both the cell lines. No activated Akt protein in 147T and 090 HPV+ oropharyngeal cancer cell lines was detected before or after E6/E7 gene silencing (data not shown).

It is well known that p53 regulates the transcriptional activation of the PTEN gene [9]. Given the fact that previous work in our laboratory has shown a marked restoration of p53 protein levels after E6/E7 repression [10], we sought to investigate whether p53-driven transcriptional activation of PTEN after E6/E7 silencing is implicated in PTEN protein stabilization in RVshRNAHN4-infected HPV16+ oropharyngeal cell lines. In this direction, we carried out real time RT-PCR to measure PTEN mRNA levels at baseline and 48 h after E6/E7 repression. Total RNA was purified from HPV+ oropharyngeal cell lines before and after infection by retroviruses expressing control shRNA or shRNAHN4 and subjected to quantitative RT-PCR analysis for PTEN, p21 and E7 genes. The tumor-suppressor p21 is a known p53 target gene [11]. Thus, induction of p21 mRNA levels is indicative of p53-driven transcriptional upregulation and p53 restoration. The shRNA-mediated inhibition of HPV16 E6 and E7 expression was associated with a slight upregulation of PTEN mRNA levels in 090 (1.45-fold) and 147T (1.22-fold) cells, whereas p21 mRNA levels were induced ~2.5-fold and ~2.0-fold, respectively (Figure 3C).

As mentioned above, western blot analysis showed no upregulation of EGFR protein levels after E6/E7 repression. We subsequently sought to determine whether phospho EGFR protein levels are affected by E6/E7 viral expression. Therefore, 090 and 147T cells treated with control shRNA (−) and shRNAHN4 (+) were subjected to immunoblotting with antibodies recognizing EGFR and phospho EGFR. As seen in Figure 4, phospho EGFR levels (Tyr845, Tyr992) were not affected as compared to control shRNA samples (Figure 3C).

### Table 1. Comparison of AQUA mean scores (with 95% CI) of 13 biomarkers between p16+ and p16− tumors

<table>
<thead>
<tr>
<th></th>
<th>p16+</th>
<th>p16−</th>
<th>P</th>
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<tbody>
<tr>
<td>EGFR</td>
<td>19.36</td>
<td>17.63</td>
<td>NS</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>27.87</td>
<td>19.2</td>
<td>0.015</td>
</tr>
<tr>
<td>β-catenin</td>
<td>27.87</td>
<td>17.2</td>
<td>0.006</td>
</tr>
<tr>
<td>MET</td>
<td>17.29</td>
<td>15.44</td>
<td>NS</td>
</tr>
<tr>
<td>ERK1/2</td>
<td>23.44</td>
<td>20.69</td>
<td>NS</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>13.44</td>
<td>4.85</td>
<td>0.005</td>
</tr>
<tr>
<td>PI3Kp110</td>
<td>33.78</td>
<td>25</td>
<td>0.035</td>
</tr>
<tr>
<td>PI3Kp85</td>
<td>31.66</td>
<td>30.92</td>
<td>NS</td>
</tr>
<tr>
<td>PTEN</td>
<td>12.61</td>
<td>8.68</td>
<td>0.009</td>
</tr>
<tr>
<td>NFkB</td>
<td>23.66</td>
<td>16.22</td>
<td>0.007</td>
</tr>
<tr>
<td>pAkt</td>
<td>19.34</td>
<td>17.47</td>
<td>NS</td>
</tr>
<tr>
<td>STAT3</td>
<td>20.02</td>
<td>13.93</td>
<td>NS</td>
</tr>
<tr>
<td>p53</td>
<td>8.67</td>
<td>11.18</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, nonsignificant.

P16+ tumors had significantly higher β-catenin (P = 0.006), E-cadherin (P = 0.015), Bcl-2 (P = 0.005), NFkB (P = 0.007), PI3Kp110 (P = 0.035) and PTEN (P = 0.0009) expression levels.

### Table 2. Comparison of mean AQUA scores (with 95% CI) of 13 biomarkers with the p16/HPV status

<table>
<thead>
<tr>
<th></th>
<th>p16+/HPV+</th>
<th>p16+/HPV−</th>
<th>p16−/HPV+</th>
<th>p16−/HPV−</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>18.1 (10.9–25.3)</td>
<td>6.3 (0–33.8)</td>
<td>23.5 (8.9–38)</td>
<td>13.6 (0–128)</td>
<td>NS</td>
</tr>
<tr>
<td>p53</td>
<td>11.3 (6.1–16.4)</td>
<td>6.3 (0–33.8)</td>
<td>8.2 (4.6–11.9)</td>
<td>9.3 (6.9–12.4)</td>
<td>NS</td>
</tr>
<tr>
<td>β-catenin</td>
<td>19 (12.8–25.2)</td>
<td>25.9 (16.5–35.7)</td>
<td>39.2 (14–64.4)</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>MET</td>
<td>15 (10.6–19.4)</td>
<td>18.7 (13.2–24.3)</td>
<td>11.5 (7.9–15.1)</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>ERK1/2</td>
<td>23.1 (11.5–34.7)</td>
<td>24.9 (13.5–36.3)</td>
<td>11.5 (7.9–15.1)</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>Bcl-2</td>
<td>4.3 (3.5–5.5)</td>
<td>4.5 (0–11.5)</td>
<td>11.4 (1.8–21)</td>
<td>25.5 (0–85)</td>
<td>0.08</td>
</tr>
<tr>
<td>PI3Kp110</td>
<td>21.1 (14.9–27.2)</td>
<td>11.9 (0–42.6)</td>
<td>35.3 (26.6–44)</td>
<td>26.7 (13.8–39.6)</td>
<td>0.022</td>
</tr>
<tr>
<td>PI3Kp85</td>
<td>25.8 (21.2–30.4)</td>
<td>14.1 (0–41.7)</td>
<td>31.9 (26.1–37.7)</td>
<td>28.6 (21.2–36)</td>
<td>NS</td>
</tr>
<tr>
<td>PTEN</td>
<td>8.1 (7.9–9.2)</td>
<td>6.5 (0–15)</td>
<td>11.3 (8.9–13.8)</td>
<td>17.8 (4.3–31.2)</td>
<td>0.004</td>
</tr>
<tr>
<td>NFkB</td>
<td>15.4 (10.2–20.7)</td>
<td>4.4 (0–20)</td>
<td>21.4 (15.9–27)</td>
<td>23.1 (9.8–36.3)</td>
<td>NS</td>
</tr>
<tr>
<td>pAkt</td>
<td>15.1 (12.4–17.9)</td>
<td>13.5</td>
<td>16.1 (11.4–20.9)</td>
<td>26</td>
<td>NS</td>
</tr>
<tr>
<td>STAT3</td>
<td>13.5 (6–21)</td>
<td>20 (9–31)</td>
<td>19 (4.2–33.8)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Three HPV+/p16+ tumors expressed significantly higher levels of E-cadherin (P = 0.003), PTEN (0.004), β-catenin (P = 0.07) and bcl-2 (P = 0.08), as well as lower levels of PI3Kp110 (P = 0.022).
results indicate that, in addition to their effects on p53 and pRb tumor-suppressor pathways, the main viral oncogenes exert their oncogenic properties by activating EGFR and reducing PTEN levels.

discussion

In clinical trials, HNSCCs have largely been regarded as a single disease entity. Recent work, however, suggests that considerable differences exist between some HNSCCs, beyond those defined by traditional clinical–pathological factors [12]. HPV represents the most validated prognostic biomarker in HNSCCs. The intensification of standard therapy in HNSCCs over time, with the use of higher total radiation dose, concurrent high-dose cisplatin and the use of combination chemotherapy before chemoradiation resulted from trials which included predominantly non-HPV associated cancers, and these morbid approaches may not be necessary for all HPV-associated oropharynx cancers. Clinical trials with HPV selection are only now being undertaken. Treatment deintensification in HPV+ HNSCC appears to be an interesting research question. Furthermore, the identification of pivotal pathways that govern tumor progression in HPV-associated tumors is likely to lead to targeted approaches to therapy. Our group demonstrated that p16 is a surrogate biomarker for biologically and clinically relevant HPV infection in oropharyngeal squamous cell carcinoma [8]. We showed that the p16 expression status is a strong prognostic indicator in oropharyngeal squamous cell carcinoma independent of the stage. In the present paper, we validate the prognostic importance of p16 in HNSCCs, and we further demonstrate its prognostic significance independent of the tumor site.
result from mutation, deletion or promoter methylation in HPV-unrelated HNSCCs [17–19]. The present report is the first to demonstrate that the E6 and E7 oncoproteins of HPV 16 downregulate the tumor-suppressor protein PTEN.

Recently, we showed that repression of HPV16 viral oncogenes E6 and E7 genes via shRNA silencing, led to a rapid restoration of p53 and pRb tumor-suppressor pathways in HPV type 16+ OSCC cell lines, respectively [5]. Previous studies showed that p53 protein can bind to a PTEN promoter and activate PTEN expression at the transcriptional level [9]. Furthermore, PTEN and p53 interact and form a complex which protects p53 from Mdm2-protein-mediated degradation [20]. Thus, it seems that PTEN autoregulates its expression, which protects p53 from Mdm2-protein-mediated degradation. Furthermore, PTEN and p53 interact and form a complex containing proteins for degradation. The MAGI proteins MAGI-2 and MAGI-3 have been shown to bind PTEN, increasing its stability [21]. Therefore, it is also possible that PTEN upregulation after E6/E7 repression could result from restoration of MAGI expression.

In addition, we found that there was no significant difference in EGFR protein levels in p16+ versus p16− HNSCCs. EGFR is a validated molecular target in HNSCCs, but the role of EGFR signaling activation in HPV+ versus HPV− HNSCCs has not been determined. To demonstrate whether the main viral oncogenes affect the expression and activation status of EGFR and downstream targets, we evaluated expression levels of key components of the pathway before and after E6/E7 gene silencing. We found that pEGFR is down-regulated and PTEN is upregulated following E6/E7 repression. Notably, Vivanco et al. recently showed that PTEN promotes the ubiquitination of activated EGFR, so we cannot formally exclude the possibility that the downregulation of phospho EGFR after E6/E7 repression is a direct effect of PTEN restoration on activated EGFR ubiquitination [22]. These results provide indirect evidence that the EGFR is one of the pathways manipulated by HPV to promote malignant transformation in host cells.

To conclude, the findings of the present study have significant therapeutic implications. First, we provide support for further study of β-catenin and Wnt signaling as a molecular target in HPV-associated HNSCCs. Second, we demonstrate that EGFR signaling activation is one of the mechanisms HPV utilizes to exert its malignant properties.

acknowledgements

The authors thank Spyros Siolos for assistance in statistics. Portions of this material were presented at the 45th Annual Meeting of the American Society of Clinical Oncology, June 2009.

funding

This study was funded by Yale School of Medicine Institutional startup funds (AP) and the Virginia Alden Wright Fund (CS).

disclosure

DR is a scientific founder and consultant for HistoRx, the Yale licensee of the AQUA patent. Beyond that, there is no conflict of interest on the part of any other authors on this work.

references

Long-term follow-up of a phase III study comparing radiotherapy with or without weekly oxaliplatin for locoregionally advanced nasopharyngeal carcinoma

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Received 10 December 2012; revised 4 March 2013; accepted 27 March 2013

Background: Previous results from our trial showed that adding oxaliplatin to radiotherapy (RT) increased survival in patients with locoregionally advanced nasopharyngeal carcinoma (NPC) at 2 years. Here, we present the data of long-term efficacy and late toxic effects.

Patients and methods: Between January 2001 and January 2003, 115 Patients with nonkeratinizing/undifferentiated locoregionally advanced NPC were randomly to receive either RT alone (n = 56) or plus concurrent oxaliplatin 70 mg/m² weekly for six cycles (n = 59).

Results: After a median follow-up of 114 months (range 18–139 months), the 5-year overall survival (OS) and metastasis-free survival (MFS) rates in the concurrent chemoradiotherapy (CCRT) group were significantly higher than those observed in the RT-alone group (OS, 73.2% versus 60.2%, P = 0.028; MFS, 74.7% versus 63.0%, P = 0.027). However, CCRT did not improve locoregional failure-free survival significantly. Subgroup analyses showed that the superiorities of CCRT mainly existed in the T3-4N0-1 stage subgroup (OS: HR = 0.394, P = 0.034). The grade 3/4 late toxic effects were similar in the two groups.

Conclusion(s): The long-term follow-up data confirms the role of CCRT as a treatment of locoregionally advanced NPC. Oxaliplatin can be considered as an alternative optional therapeutic regimen for these patients due to its high efficiency and low toxic effect.

Key words: nasopharyngeal carcinoma, concurrent chemotherapy, oxaliplatin, radiotherapy