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Biological Features of Human Papillomavirus-related Head and Neck Cancers Contributing to Improved Response

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Abstract

Head and neck squamous cell carcinomas (HNSCC) are the sixth most common malignancy globally, and an increasing proportion of oropharyngeal HNSCCs are associated with the human papillomavirus (HPV). Patients with HPV-associated tumours have markedly improved overall and disease-specific survival compared with their HPV-negative counterparts when treated with chemoradiation. Although the difference in outcomes between these two groups is clearly established, the mechanism underlying these differences remains an area of investigation. Data from preclinical, clinical and genomics studies have started to suggest that an increase in radio-sensitivity of HPV-positive HNSCC may be responsible for improved outcomes, the putative mechanisms of which we will review here. The Cancer Genome Atlas and others have recently documented a multitude of molecular differences between HPV-positive and HPV-negative tumours. Preclinical investigations by multiple groups have explored possible mechanisms of increased sensitivity to therapy, including examining differences in DNA repair, hypoxia and the immune response. In addition to differences in the response to therapy, some groups have started to investigate phenotypic differences between the two diseases, such as tumour invasiveness. Finally, we will conclude with a brief review of ongoing clinical trials that are attempting to de-escalate treatment to minimise long-term toxicity while maintaining cure rates. New insights from preclinical and genomic studies may eventually lead to personalised treatment paradigms for HPV-positive patients.

Keywords

Biology; DNA repair; head and neck cancer; HPV; radiosensitivity

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Introduction

Head and neck squamous cell carcinomas (HNSCC) have long been recognised to arise as a result of environmental exposures including tobacco and alcohol. However, in the past 15 years, human papillomavirus (HPV) positivity has been recognised as an aetiological factor for a substantial and increasing subset of these tumours [1]. Since the discovery of a separate viral aetiology of HNSCC, multiple groups have shown significantly improved outcomes after treatment for HPV-positive tumours when compared with HPV-negative tumours [2–4]. Most of these series have involved patients treated either with radiotherapy or chemoradiotherapy, suggesting that HPV-positive tumours may be particularly sensitive to ionising radiation. HPV positivity is also a favourable prognostic factor in anal cancers, which are also typically treated with definitive regimens of chemoradiation with generally favourable outcomes [5–11]. Cervix cancers are nearly 95–100% associated with HPV infection, but nevertheless some series show that HPV-negative or certain HPV subtypes are associated with worse outcome [10,12–14]. Taken together, HPV positivity across multiple disease sites results in a favourable outcome to chemoradiation, and strongly suggests a particular cellular sensitivity to DNA-damaging agents. Further *in vitro* characterisations of HPV-associated cell lines has suggested increased radiosensitivity [15,16].

Potential mechanistic explanations for improved outcomes will be the focus of this review. HPV-positive HNSCCs have some molecular similarities, but many differences compared with HPV-negative HNSCCs (Table 1). In some senses, HPV-positive HNSCCs can seem molecularly more similar to HPV-positive tumours in other parts of the body (anal and cervical cancer) than HPV-negative HNSCC [17–19]. We will first review the key molecular and genetic differences between HPV-positive and HPV-negative tumours that may underlie some of the differences in sensitivity. Second we will summarise research investigating the underlying mechanisms of improved radiation response with a special focus on three main areas of investigation: hypoxia, the immune response and the DNA damage response (DDR) (Fig. 1). Historically, the efficacy of ionising radiation has been attributed to the four Rs of radiobiology: repair of DNA damage, re-oxygenation, re-distribution in the cell cycle and repopulation. HPV oncogenesis affects each of these components: it hijacks the cellular machinery for DNA repair, HPV-positive tumours display unique kinetics of hypoxia during radiation treatment, HPV alters the cell cycle distribution of infected cells by regulating checkpoint mediators and it induces rapid cellular proliferation [20]. Third, we will review other possibilities for improved outcomes rather than increased sensitivity, such as phenotypic differences between HPV-positive and HPV-negative tumours. Finally, we will discuss current efforts to de-escalate treatment and minimise toxicity for patients. A more precise understanding of the mechanism of improved response will ultimately help guide rational de-escalation trial design.

Genomic and Molecular Differences between Human Papillomavirus-positive and -negative Tumours

The HPV genome contains two oncogenes, E6 and E7, which inhibit p53 and Rb, respectively, and are thought to be primarily responsible for viral oncogenesis. Although

most HPV-negative HNSCCs have a *TP53* mutation, HPV-positive HNSCCs rarely do because E6 expression disables *TP53* instead [21,22]. Nearly half to three-quarters of HPV-negative HNSCCs have mutated *TP53* [21]. *CDKN2A*, the gene coding for the protein p16, is inactivated in an overwhelming portion of HPV-negative tumours. About 30% of HNSCC have homozygous deletions of *CDKN2A*; 10–20% have loss of function mutations and a similar fraction have inactivating epigenetic alterations [22]. Degradation of Rb by E7 drives overexpression of p16. Thus, high expression of p16 is a surrogate marker for HPV-driven oncogenesis and has also shown to be a prognostic factor for radiotherapy outcome [23, 24]. Mutations in Rb itself are more likely to appear in HPV-positive tumours, although such occurrences are rare, probably due to a lack of selective pressure in the presence of E6 [22].

Presumably as a response to viral infection, HPV-associated tumours display evidence of induction of a component of innate immunity known as the apolipoprotein B mRNA editing enzyme catalytic polypeptide-like (APOBEC) family of proteins. This is a class of editing enzymes that change cytosine to uracil by deamination, thereby hypermutating and inactivating viral DNA. The replacement of the uracil commonly results in a mutation to thymine or guanine. The mutation signature of these enzymes is frequently found in virally transformed cancers and it has been found to be especially active in HPV-positive HNSCC [25]. Potentially as a consequence of high APOBEC activity, two specific codons (E542K and E545K) within the helicase domain of *PIK3CA* are frequently mutated in HPV-positive HNSCC due to C>T transitions. In HPV-negative tumours, *PIK3CA* mutations are spread throughout the gene and are significantly less frequent (12% versus 33%) [25]. This APOBEC pathway may be a driving mechanism behind HPV-associated transformation, but whether activation of this pathway affects therapeutic sensitivity is unknown.

On a gene-expression basis, HNSCC has been divided into four subtypes – basal, mesenchymal, atypical and classical – as first described by Chung *et al.* in 2004 [26]. These subtypes were confirmed by Walter *et al.* in 2009 [27] and by TCGA in 2015 [17]. HPV-positive tumours tend to be enriched in the atypical subtype. Keck *et al.* [28] recently classified two HPV-positive and three HPV-negative HNSCC subtypes based on a large set of expression data that correlate with the traditional four subtypes but also have some distinguishing characteristics. Additional replication of this latter subtyping may be needed before it is widely accepted.

Common copy number changes in HNSCC include gains of the 3q, 7p and 11q regions as well as losses of 3p, 9p and 14q [27]. Some of these changes appear across all expression subtypes, whereas others are exclusive to one or multiple expression subtypes. Many copy number alterations are shared between both HPV-positive and HPV-negative tumours (Table 1). Interestingly, none of the atypical subtype samples show gains of the 7p region, where epidermal growth factor receptor is located, whereas the other three do to some degree. Additional differences in HPV-positive tumours include frequent homozygous deletions in the *TRAF3* gene involved in innate immunity and frequent deletion of 11q containing the *ATM* gene [22].

DNA Repair-mediated Mechanisms for Increased Therapeutic Sensitivity

The HPV family has evolved to exploit the host DDR machinery in order to avoid eradication as well as to promote its propagation. The papillomavirus proteins E1 and E2 are essential for viral genome replication. HPV E1, a DNA helicase, binds directly to origins of HPV DNA replication, mediated by the regulatory HPV protein E2 [29]. E1 and E2 co-localise to sites of viral DNA replication with γ H2AX, phosphorylated ATM and p53, thereby activating the DDR at these sites to facilitate viral replication [30]. E7 ensures that the host cell continues through the cell cycle by phosphorylating proteins that lock up E2F transcription factors in order to override arrest signals brought by an activated DDR. E6 decouples apoptosis from the DDR by p53 degradation to keep the host cell alive in spite of the increase in genomic instability due to unchecked cell cycle progression. Attenuation of these DDR proteins by HPV may interfere with the host's normal DDR after exposure to therapeutic radiation.

Hence, one of the most direct mechanisms for an increased response to radiation in HPV-positive tumours may be due to changes in the DDR. HPV-positive HNSCC cell lines have been shown on average to be more sensitive to radiation as measured by colony survival assays [16]. These findings were independent of either apoptosis or G1 arrest, suggesting defects outside of cell cycle regulation are responsible for sensitivity. These same cell lines were found to have increased residual γ H2AX/53BP1 foci and a persistent G2 arrest after 2 Gy. Foci counts negatively correlated with survival in HPV-positive cell lines, whereas such a relationship did not hold HPV-negative cell lines. Beyond comparisons of groups of cell lines, the Kimple group has made the isogenic comparison of an immortalised tonsillar epithelial cell line with and without expression of E7 and found delayed kinetics of double-strand break repair as assessed by foci counting. Likewise in a transgenic mouse model wherein HPV16 E7 is expressed in squamous epithelium, γ H2AX foci resolution is delayed after radiation compared with isogenic controls [31]. In addition, E6 expression was shown to radiosensitise oropharyngeal cancer lines and increase the frequency of programmed cell death after irradiation [32]. These experiments provide strong preclinical evidence of an intrinsic cellular radiosensitivity that is secondary to impaired double-strand break repair.

Although the precise mechanism of impairment in double-strand break repair remains poorly understood, one hypothesis is that E7 inhibits Rb and its paralog pocket proteins p107 and p130, which have known roles in canonical non-homologous end joining, independent of their function in cell cycle regulation and suppression of E2F transcription [33].

Furthermore, loss of Rb has been shown to be associated with genomic instability in response to DNA damage with observed increases in chromosomal instability and resultant aneuploidy and polyploidy [34]. Finally, recent evidence has shown that Rb binds to p53 binding protein 1, linking Rb function directly to the DDR [35]. Loss of Rb delays resolution of γ H2AX foci much like a trend seen in an aforementioned study concerning *in vitro* HPV-positive HNSCC lines. It may very well be that the inactivation of Rb, a well-established function of the HPV oncoprotein E7, contributes to the disruption of DNA repair mechanisms, particularly via inhibition of non-homologous end joining. Further evidence implicating the importance of E7 interactions with the DDR in HPV-driven oncogenesis originates from a murine model of Fanconi anaemia. Expression of the E7 in Fanconi

anaemic-deficient mice induces not only oncogenesis, but also γ H2AX and 53BP1 nuclear foci, an effect that is recapitulated by inactivation of Rb, p107 and p130, further implicating the E7-Rb pathway in DNA damage repair [36].

Other potential hypotheses include a direct effect of expressed p16 in HPV-positive HNSCC, as p16INK4a was found to suppress RAD51 foci formation and increase the frequency of micronuclei [37]. Another hypothesis implicates cyclin D1 (CCND1), another protein involved in cell cycle regulation that has been found to have an independent role in DDR. CCND1 was found to bind directly to RAD51 and its interaction is induced by radiation [38]. Depletion of CCND1 impaired recruitment of RAD51 to damaged sites, impeded HR and increased sensitivity to radiation. As HPV-positive tumours highly express p16, which suppresses CCND1, and as HPV-negative tumours tend to exhibit amplifications in CCND1, this effect may also play a role in the observed radiosensitivity of HPV-related HNSCC.

In summary, the above observations indicate that host DNA repair mechanisms are hijacked to ensure fidelity of viral DNA replication at E1/E2-associated replication foci. Meanwhile, host genomic repair and cell cycle regulation mechanisms seem to be suppressed, primarily via oncoproteins E6 and E7 and effects mediated by p53, Rb and p16ink4a pathways. This probably results in increased intrinsic radiosensitivity due to an impaired DDR.

Partial TP53 Inactivation as a Mechanism for Increased Sensitivity

Broadly, p53 is a transcription factor that regulates the expression of hundreds of downstream targets to facilitate stress responses in a cell. In response to double-strand breaks caused by ionising radiation, p53 is stabilised and activated. p53 induces a G1 arrest after irradiation to prevent damaged DNA from being replicated in S phase. Apart from cell cycle arrest, p53 has been shown to detect and bind regions of damaged DNA as well as interact with DNA repair proteins such as RAD51 and BRCA1. Finally, if the amount of DNA damage is overwhelming or irreparable, p53 can induce apoptosis [39]. p53 missense mutations are clustered in the DNA binding domain of the protein, whereas there is a more equal distribution of nonsense and frameshift mutations [21]. As p53 is part of a tetramer, these mutations are thought to have a dominant negative effect on its activity. In cells that make use of p53-mediated apoptosis, these loss of function mutations may lead to radioresistance, but the use of this pathway in epithelial cells is controversial. *TP53* mutations are rare in HPV-positive tumours because degradation of p53 by E6 HNSCC negates the selection of p53 mutations in this group.

Kimble *et al.* [15] compared four HPV-positive cell lines against four HPV-negative cell lines and found HPV-positive lines to have a robust increase in apoptosis and caspase activity 24 h after 4 Gy radiation, whereas no increase was seen in HPV-negative cell lines. Expression data on these same lines revealed HPV-positive samples to have a significant increase in activation of p53 and genes in its pathway after irradiation as compared with HPV-negative samples. Knockdown of remaining p53 by siRNA in HPV-positive cell lines increased colony formation, implying low levels of p53 could be activated by radiation. Along these lines, when Pang *et al.* [32] explored the radiosensitisation of HNSCC by E6, they found that the isoform responsible, E6*I, did not completely inhibit p53 activity. They

postulated that in HPV-positive tumours, E6*I may activate a dormant apoptotic pathway upon irradiation [32]. This possibility is supported by the finding that E6*I stabilises procaspase-8, an apoptotic initiator [40]. Further investigation is needed in this area as the role of p53 in radiation-induced apoptosis in epithelial tumours remains controversial.

Immune-mediated Mechanisms

There has been significant investigation into whether the presence of viral proteins, namely E6 and E7, in HPV-positive tumours elicits a stronger immune response and contributes to an improved clearance of tumour. HPV-positive tumours transplanted into immunocompromised mice grew more rapidly post-irradiation compared with HPV-negative xenografts [41]. When the same mice underwent adoptive transfer of immune cells, HPV-positive tumours were cleared more robustly after chemoradiation than their HPV-negative counterparts. The frequency of T cells specific to the E7 protein has been shown to be significantly higher in HPV-positive tumours compared with HPV-negative tumours or healthy controls [42]. Likewise, HPV-positive tumours are more likely to be seropositive for antibodies against HPV virus-like particles, E6, and E7; the presence of these antibodies correlates with improved survival [43,44]. A few studies have shown elevated expression of immunosuppressive markers in HPV-positive tumours. T cells from HPV-positive tumours express higher levels of PD-1, which inhibits their proliferation and effector functions, but still correlates with improved prognosis [45]. Furthermore, PD-L1 expression is higher in HPV-positive tumours as opposed to HPV-negative HNSCC tumours, which may play a role in immune resistance [46]. It is thought that PD-1 status reflects a previous immune response that can be reactivated during treatment. In fact, radiation has been found to reduce the overexpression of CD47, a marker of self, in oropharyngeal cancers in a dose-dependent manner [47]. Although aspects of the above data are conflicting, taken together they implicate the immune response as an important factor in the determination of the response to radiotherapy in HPV-positive disease.

Hypoxia

Ionising radiation produces radicals that are fixed by oxygen and cause DNA damage. In hypoxic conditions, there is less oxygen for fixation and thus less DNA damage as a result. Tumours in hypoxic conditions can have up to a three-fold increase in radiosensitivity compared with those in normoxic conditions [48]. Furthermore, multiple clinical studies have shown worse overall survival and locoregional control with hypoxic head and neck tumours compared with their normoxic counterparts [49,50]. As hypoxic tumours are known to be more radioresistant, it was postulated that HPV-positive tumours may be less hypoxic as compared with HPV-negative tumours, contributing to their enhanced radiation response [50]. Hypoxic volumes of HNSCC tumours were imaged by FAZA positron emission tomography/computed tomography in patients of the DAHANCA 24 trial. Although patients with non-hypoxic tumours exhibited enhanced survival, no significant difference in distribution of hypoxia was found between HPV-positive and HPV-negative tumours [51]. Similarly, gene expression data from HPV-positive and HPV-negative HNSCC biopsies of DAHANCA 5 showed no differences in the frequency of hypoxic tumours as classified by a profile of 15 hypoxia-related genes [52].

This suggested that there was no baseline difference in hypoxia between HPV-positive or HPV-negative tumours and that this alone could not explain differences in sensitivity between the two types of cancer. Furthermore, analysis of the DAHANCA 5 trial showed a benefit from nimorazole, a hypoxic modifier, was limited to p16-negative HNSCC tumours, with minimal efficacy in p16-positive tumours [53]. These authors speculated that there may be a difference in hypoxic stem cells between HPV-positive and HPV-negative tumours or that HPV-positive tumours do not thrive in hypoxic microenvironments. However, we have recently noted that hypoxia resolves early during the course of therapy in a subset of HPV-positive tumours, which may equally explain the lack of benefit of a hypoxic radiosensitiser in patients with early resolution [54]. The role of hypoxia in these persistently positive tumours remains to be fully elucidated.

Phenotypic Differences Unrelated to Therapeutic Sensitivity

It must be acknowledged that there may be alternative explanations for improved outcomes in HPV-related patients rather than an increased sensitivity to chemoradiation. HPV association in cancer tends to be relatively exclusive of a smoking history in patients, which inevitably impacts overall patient health. Second, outcomes in HPV-associated HNSCC are better than HPV-negative HNSCC in patients who undergo primary surgical therapy, suggesting that HPV association could be a prognostic instead of a predictive variable with regard to radiation sensitivity. A key caveat is that most oropharyngeal HNSCC patients treated with upfront surgical resection also receive adjuvant radiation therapy, clouding this interpretation [55].

Furthermore, there are phenotypic differences between HPV-positive and HPV-negative tumours. For instance, p16-positive oropharyngeal carcinomas also display a less locally invasive phenotype, which may account for improved outcomes. p16Ink4a overexpression in oral cavity tumour cell lines decreases tumour invasiveness by affecting extracellular matrix remodelling and expression of matrix metalloproteinases potentially accounting for the observation that p16-positive tumours are less locally invasive [56]. Additionally, in a cohort of primarily p16-positive oropharyngeal cancers that were surgically managed, nodal extracapsular extension, unless the node was completely obliterated, was not associated with poorer outcomes, as has been well established in HPV-negative HNSCC, suggesting a less locally aggressive phenotype in HPV-positive disease [57–59]. Finally, HPV-positive oropharyngeal carcinomas are more commonly seen in young, otherwise healthy, men who are better able to tolerate treatment and have a longer life expectancy [60].

Clinical Trial Investigating Therapeutic De-escalation

Patients with HPV-related oropharyngeal carcinoma have superior outcomes in terms of locoregional control and survival compared with those with HPV-negative disease. The current standard of care, however, offers the same treatment approaches to patients regardless of HPV status. For patients with HPV-positive disease, who are younger with fewer comorbidities, the long-term sequelae of chemoradiation include xerostomia, dysphagia, soft tissue fibrosis and ototoxicity, all of which can significantly and permanently affect quality of life. Clinical trials are now underway to de-escalate definitive treatment in

HPV-positive patients in an effort to preserve excellent clinical outcomes while minimising toxicity. The various ongoing approaches will be discussed below, including: reduction of radiation dose, the use of cisplatin alternatives, adaptation of radiation according to response to induction chemotherapy and the integration of minimally invasive surgery into the multimodality approach. Presumably, alterations in DNA repair pathways mediated by HPV probably account for the increased radiosensitivity that will allow some of these approaches to succeed.

Although the widespread adoption of intensity-modulated radiation therapy has served to reduce some toxicities by improved avoidance of uninvolved adjacent structures, there remains much room for improvement. As such, many ongoing clinical trials are evaluating outcomes with reduced radiation doses given the observed sensitivity of HPV-positive disease to chemoradiation (NRG HN002, NCT0153099, NCT01088802, NCT01891695) [61]. Proton radiotherapy is also being investigated at some centres as a method to improve the precision of treatment and spare surrounding organs at risk (NCT01893307). Importantly, the radiosensitivity of HPV-positive tumours is quite heterogeneous, both *in vitro* and *in vivo*. It will be important to find biomarkers to identify which HPV-positive tumours can safely be deescalated. The hypoxia tracer F-MISO allows selective dose de-escalation for tumours without significant hypoxia to a dose of 60 Gy on the basis of FMISO positron emission tomography imaging before and early in the course of treatment [54]. It should be noted that most of these studies use only modest dose de-escalation from 70 Gy to 60 Gy.

Another potential method for de-intensification of treatment is to substitute a less toxic systemic agent for cisplatin, the standard radiosensitiser used in HNSCC. Cetuximab, a partially humanised antibody for the epidermal growth factor receptor, is Food and Drug Administration approved for the treatment of HNSCC [62]. RTOG 1016 and the De-ESCALaTE trial are currently assessing radiotherapy with concurrent cetuximab versus cisplatin in locally advanced HPV-positive disease. Of note, recent data have suggested inferior outcomes after bio-radiotherapy with panitumumab or cetuximab in combination with radiotherapy compared with cisplatin, which has somewhat dampened enthusiasm for this approach [63,64].

It is well established that the response to induction chemotherapy can predict the response to radiation therapy. ECOG 1308 (ASCO 2013) attempted to modulate the use of definitive chemoradiation for HPV-positive patients based on their response to induction chemotherapy and presumed associated sensitivity to radiotherapy. This study has shown acceptable rates of progression-free survival in patients who achieved a complete response to induction paclitaxel, cisplatin and cetuximab who went on to receive definitive radiation to the de-escalated dose of 54 Gy with concurrent cetuximab. Similarly, Mount Sinai School of Medicine is currently conducting a randomised phase III trial comparing induction chemotherapy followed by chemoradiation to standard dose (70 Gy) with concurrent carboplatin versus chemoradiation to a de-escalated dose of 56 Gy with concurrent cetuximab and carboplatin (NCT01706939).

Trans-oral robotic surgery is a minimally invasive approach that is currently being explored in combination with radiation and systemic approaches to allow for postoperative radiation dose de-escalation in a subset of patients (ECOG 3311, NCT01898494, PATHOS trial NCT02215265, ADEPT trial NCT01687413). The premise behind this approach is that a minimally invasive surgical approach with postoperative radiotherapy will lead to reduced long-term toxicity compared with upfront chemoradiation. A caveat to this method is that those patients with high-risk features on pathological examination, such as extra-capsular extension, will still require chemotherapy – and that these patients in essence will receive escalated therapy (WashU, NCT01687413).

Conclusion

Although the mechanistic basis of improved outcomes of HPV-positive HNSCC after irradiation remains unclear, we have reached the end of the beginning of the narrative over the past decade. The genetic landscapes of HPV-positive and HPV-negative patients have key distinguishing features as reaffirmed by recent TCGA data, and these distinct backgrounds result in divergent survival rates as seen in the clinic. Most of the recent preclinical data suggest that HPV-positive cells are more sensitive to IR *in vitro*. HPV is known to hijack the host DDR to facilitate viral replication and there is emerging evidence that this may lead to increased sensitivity to IR *in vitro*. In addition, differences in the immune response and changes in the kinetics of hypoxia may also play a role in improved outcomes. As our understanding of HPV-positive tumours and their responses improve in the near future, the ability to personalise therapy based on the biology of each individual tumour will allow for new treatment paradigms for these patients.

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Statement of Search Strategies Used and Sources of Information

To find relevant literature articles, articles discussing the response of human papillomavirus (HPV)-positive head and neck squamous cell carcinoma (HNSCC) to treatment were found using PubMed and Google Scholar with key words such as head and neck cancer, HNSCC, HPV, radiation, P16, survival and sensitivity. Abstracts were skimmed for relevance and reviews on related topics were read for additional references that may have had data relevant to our discussion. Articles on subjects discussed in this review were then searched for using the same databases and combinations of the aforementioned key words together with DNA repair, DNA damage, genomics, invasiveness, E6, E7, immune response, hypoxia, apoptosis, Rb, p53 and CCND1.

Fig. 1.

Three main areas of focus for observed improved response to radiation therapy seen in human papillomavirus (HPV)-positive patients, namely: disruption of DNA repair, enhanced immune response and rapid intra-treatment resolution of hypoxia.

Table 1

Prevalence of genetic alterations in notable genes between human papillomavirus (HPV)-negative and HPV-positive samples

	HPV–	HPV+
ATM	Common	Very Common
CCND1	Very Common	Rare
CDKN2A	Most	Rare
EGFR	Common	Rare
FGFR1	Common	Rare
NOTCH1	Very Common	Common
p53	Most	Rare
PIK3CA	Common	Very Common
Rb	Rare	Rare
TRAF3	Rare	Very Common

Rare defined as < 5%, Common 10–20%, Very Common 20–50%, Most > 50%.