Review

An Epidemic of Oropharyngeal Squamous Cell Carcinoma (OSCC) Due to Human Papillomavirus (HPV) Infection and Aspects of Treatment and Prevention

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Abstract. *In Europe and the US the incidence of oropharyngeal squamous cell carcinoma (OSCC), where tonsillar and tongue base cancer dominate, has increased and we propose this is due to an epidemic of human papillomavirus (HPV) infection. We base our hypothesis on two main findings. Firstly, in the past decade numerous reports have shown the presence of HPV in OSCC, and that HPV-positive OSCC has a significantly better response to therapy than HPV-negative OSCC. As a consequence of these findings, the International Agency for Research against Cancer (IARC) has acknowledged HPV, in addition to smoking and alcohol, as a risk factor for OSCC. Secondly, we and others have demonstrated a considerable and significant increase in the proportion of HPV-positive tonsillar and tongue base cancer/OSCC in recent decades. In conclusion, we suggest that the increased incidence of OSCC is due to an HPV epidemic. In addition, since patients with HPV-positive OSCC have a better response to therapy than those with HPV-negative OSCC, we put forward that it is important to individualize therapy on the basis of HPV status and other markers in order to obtain optimal survival with the least side-effects. Finally, we suggest that the possibility to vaccinate against HPV-positive OSCC should be examined.*

Although use of preventive vaccines against some human papillomavirus (HPV) types, especially HPV-16 and -18, has been introduced with the goal of abrogating around 70% of all HPV-induced cervical cancer, we should remember that HPV is also associated with other cancer types such as head and neck and anogenital cancer (1-3). In particular, we would like to draw attention to what is believed to be an HPV-induced epidemic increase of oropharyngeal squamous cell carcinoma (OSCC). OSCC is a subgroup of head neck squamous cell carcinoma (HNSCC), where the International Agency for Research against Cancer (IARC) has acknowledged HPV as a risk factor, together with the traditional risk factors of smoking and alcohol and betel chewing (4-7). The incidence of HNSCC and OSCC varies widely; both HNSCC and OSCC are more common in men than in women, and in India and Latin countries as compared to the US and Northern Europe (8). In the past decade, however, several reports have shown that the proportion as well as the incidence of HPV-positive OSCC cases has increased, and in addition, that patients with HPV-positive OSCC have upon treatment a better 5-year disease-specific survival than those with HPV-negative OSSC (9-18).

In recent years, since HNSCC, including OSCC, has a very poor prognosis, treatment for these types of cancer has been intensified, with induction chemotherapy, hyperfractionated radiotherapy, and sometimes the use of EGFR inhibitors (5, 17, 19). This has led to many more acute and chronic adverse side-affects such as difficulties in eating, speaking and breathing. In this context, we propose that we are encountering an epidemic of OSCC due to HPV infection, and that many patients with a better prognosis may potentially risk being overtreated. We find it important to find ways of better individualizing OSCC patient therapy and also to consider the possibility of vaccinating against HPV-positive OSCC.

Human Papillomavirus (HPV)

There are numerous HPV types, now approaching 200 different types. Many types are found in the skin, while others are found in mucous tissues. In addition, some HPV types, the so-called high-risk (HR) types, are often associated with cancer, while other so-called low-risk (LR) types are usually

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not. The association of different HR HPV types with cervical, anogential and head and neck cancer is convincing (3). All HPVs are small DNA viruses with a genome of around 8 kb that consists of double-stranded circular DNA, and which is enclosed in a 52-55 nm viral capsid. The genome is arbitrarily divided into three regions; the early and late regions, and the non-coding control region (NCCR). The early region encodes the E1-E2, E4-E7 proteins responsible for gene regulation, replication, pathogenesis and transformation (3). In HR HPVs, E6 binding and degradation of p53, and E7 binding and inhibition of the retinoblastoma protein (Rb) result in deregulation of cell cycle control (3). The late region encodes for L1 and L2, the major and minor viral capsid proteins respectively. The major capsid protein L1 can self-assemble into virus-like particles (VLPs), and such VLPs are used in the presently available vaccines against HPV infection (1-3).

Detection Methods for HPV

PCR technology is now frequently used for the detection of HPV DNA (19-22). Screening for HPV can be performed using general PCR primers for HPV, allowing detection of several HPV types, thereafter followed by type-specific PCRs or sequencing (20-22). In addition, in order to assess the quality of the DNA, a PCR of a control cellular gene is used in parallel. Other more sensitive methods have also been developed such as one developed by Schmitt *et al*. who used a sensitive bead-based multiplex method set up for many different HPV types and where the HPV PCR products are coupled to type-specific probes on beads and analyzed by Luminex (23).

However, when using very sensitive techniques for HPV DNA detection, it is important to also assay for the biological activity of HPV. Thus, examining the presence of E6 and E7 mRNA by RT-PCR can be performed (13). In addition, $p16^{INK4a}$ immunohistochemistry (IHC) can also be used to assay for biologically active HPV, since there is an association between the presence of HPV and $p16^{INK4a}$ overexpression (24-26). Moreover, in some laboratories, $p16^{INK4a}$ IHC is used as a substitute marker for HPV. Nevertheless, it must be noted that the correlation between presence of HPV and $p16^{INK4a}$ overexpression is not absolute since some tumours express $p16^{INK4a}$ in the absence of HPV (24).

HPV and OSCC

In the past, HPV DNA was suggested to be present in around 25% of HNSCC (6, 16, 17). Over time, however, numerous reports have demonstrated that HPV is definitely more common in OSCC as compared to other types of HNSCC, with 45-100% of the tumours reported to be HPV-positive (4-6, 11-19). The observed variation was suggested to be due to the material available, where older material was more difficult to test, or to differences in the sensitivities of the utilized techniques (17). Eventually, however, as the techniques became more sensitive, it was also shown that both the country and the time period at which the material was collected played a role (4-6, 10-17).

In many studies, OSCC cases are presented as one group, but there are studies that have analysed for the presence of HPV in separate locations of the oropharynx, especially for tonsillar and base of tongue cancer, which together account for 90% of all OSCC cases (6, 19). In such studies, the proportion of HPV-positive tonsillar cancer is sometimes slightly higher than that in base of tongue cancer (12, 27). There are as yet, no separate reports on the proportion of HPV positive OSCC cases at locations outside the tonsil and tongue base.

Nevertheless, as the presence of HPV in OSCC became apparent, numerous studies followed characterizing HPV-positive OSCC (4-6, 10-17). HPV type 16 was shown to dominate (around 90%) in OSCC, while other HPV types (*e.g.* HPV-31,-33,-58,-59,-62 and -72) occurred more occasionally (14, 28). The fact that E6 and E7 mRNA expression, as well as $p16^{INK4a}$ up regulation was observed in HPV-positive OSCC suggested that HPV was biologically active in the tumours (13, 24). The latter was an indicator of active E7, which by binding and inactivating the Rb pathway activated the cell cycle and in this way induced overexpression of $p16^{INK4a}$ (3, 24-26). In addition, HPV-positive OSCC was often less differentiated and more aneuploid than HPV-negative OSCC (29). Moreover, by comparative genomic hybridization, HPV-positive tonsillar cancer often presented chromosome 3q amplification, often found in HPV-positive cervical and vulvar cancer, thus again supporting the tumourigenic role of HPV in OSCC (30).

In summary, there are different types of OSCC, one group primarily depending on smoking and alcohol another depending mainly on HPV infection and combined aetiologies may also exist.

OSCC and HPV and Response to Treatment

It has been shown patients with HPV-positive OSCC have a better prognosis than those with HPV-negative cancer (4-6, 10-18). More specifically, patients with HPV-positive OSCC had a better 5-year survival upon treatment than those with HPV-negative OSCC *i.e.* 70-80% *vs.* 25-40% respectively, and this was independent of age, gender, tumour stage and grade of differentiation, or ploidy (4-6, 10-18). It was also shown that patients with HPV-positive OSCC that were never smokers had a better prognosis than those who were smokers (13, 15). This could be due to HPV inducing an immune response, which is abrogated by smoking. Alternatively, smoking together with the presence of HPV may induce another tumour category, with additional genetic alterations, as is suggested in several reports (13, 15-18).

Unfortunately, patients with OSCC are often treated when their tumours are large, since small tumours do not present symptoms and are thus rarely detected. In the past, treatment with intention to cure implied surgery and radiotherapy, with the intent to cause as little damage as possible and if this was not possible palliative treatment was given to limit symptoms (6, 19). However, in HNSCC, including OSCC, survival was poor and overall 5-year survival was around 25% (6, 19) and despite similar histology and stage, and treatment, it was difficult to predict clinical outcome.

To improve survival in HNSCC, in the past decade, treatment to cure has been intensified, and induction chemotherapy, followed by intensified fractionated radiotherapy, surgery, and EGFR inhibitors may be applied (16). However, intensified treatment results in more acute and chronic side-effects, such as dryness of the oral cavity with difficulty in eating, speaking and breathing. Moreover, changes in treatment have not taken into consideration that the incidence of HPV-positive OSCC with a better clinical outcome than HPV-negative OSCC is increasing. Hence, both predictive and prognostic markers would be of significant clinical value to enable individualized treatment of OSCC and to obtain optimal survival with the least side-effects (4-6, 10-18).

An HPV-induced Epidemic of OSCC

Reports from the US and many European countries show an increase in the incidence of OSCC, tonsillar cancer and base of tongue cancer and this has paralleled reports on that the proportion of HPV-positive OSCC cases has increased (9-12, 16, 17, 31-35). We suggest that this is not due to the quality of the samples assayed, since this has been validated, nor to the use of more sensitive techniques since the same techniques have been used when studying OSCC over time (9, 12, 27). Instead, we propose that we are encountering an epidemic of OSCC caused by HPV infection.

Following the incidence of tonsillar and base of tongue cancer in Sweden, from 1970 to 2006, and using the Swedish Cancer Registry we were able to demonstrate a considerable increase in both tumour types over time (9, 12, 27, 34). In two studies from 2006 and 2009 respectively, of which the second was a follow up of the first, we identified over 635 patients, with 335 available samples in the Stockholm area and using the same methodology in both studies (9, 12). In 2006, we found that the proportion of HPV-positive cancer cases increased significantly ($p<0.001$) from 23% in the 1970s to 68% between 2000-2002 (9). Moreover, there was a significant ($p<0.01$) continued increase in the proportion of HPV-positive tumours from 2000 to 2007, and between 2006-2007, 93% of all tonsillar cancer cases in the Stockholm region were HPV-positive (12). As early as 2006, we postulated that HPV infection was responsible for the increase in tonsillar cancer (9). In 2009, when making a population-based estimation, we found that the incidence rate of HPV-positive tumours doubled each decade between 1970-2007, resulting in a 7-fold increase during the study period, while in the numbers of HPV-negative tumours declined (12).

In a similar way, using the Swedish Cancer Registry and the same methodology, we showed that between 1998-2007, there was a significant increase ($p<0.05$) of HPV-positive base of tongue cancer from 58% in 1998-1999 to 84% in 2006-2007 (27).

From 2007 to 2010, there are several reports of an increase in the proportion of HPV-related OSCC cases and the proposition of a possible epidemic of HPV-associated cancer (9-12, 16, 17). It was also suggested that the increase in OSCC incidence occurs mainly in men (16), however, we have also observed an increase in women (9). Nevertheless, since the numbers of women with OSCC are limited, it has been more difficult to identify significant changes in women.

The reason for the increase in HPV-positive OSCC has been suggested to depend on change in sexual behaviours *e.g.* increased oral sex, as well as increasing numbers of sexual partners (35, 36). There is also a statistically significant association between HPV-positive tonsillar cancer and early sex debut and the number of oral or vaginal partners (36, 37). Notably, it has recently been shown among young individuals (below 20 years of age) that also open-mouthed kissing may be associated with oral HPV infection (37).

In summary, on the basis of the present data, we suggest that we are encountering an epidemic of mainly sexually transmitted HPV-induced OSCC.

An HPV-induced Epidemic of OSCC and Consequences for Treatment

The fact that HNSCC (and OSCC) treatment is intensified with induction chemotherapy, hyperfractionated radiotherapy and EGFR inhibitors, with severe side-effects, while the incidence of HPV-positive OSCC, which has a better response to therapy, is increasing, deserves special attention. Within a decade, HPV-positive OSCC may account for half of all cases of HNSCC, at least in Sweden, and possibly also in the US and other Northern European countries. It is debatable if patients with HPV-positive OSCC who are often younger and have a better prognosis than HNSCC patients in general and patients with HPV-negative OSCC, should receive this intensified therapy (4, 5, 16-18, 38). This therapy results in more acute and chronic side-effects such as oral dryness, difficulty in swallowing, talking, and sometimes necrosis of the jawbone, and it is also very costly. It is likely that it will result in considerable unnecessary side-effects for the growing group of HPV-positive OSCC patients. There is, therefore, an urgent demand to be able to identify which patients require, or do not require, intensified treatment in order to obtain optimal survival and quality of life.

Reports have been published, and studies are on-going with the aim of assessing which molecular biomarkers, *e.g.* $p16^{INK4a}$ and p53 besides HPV in OSCC can be of use to predict clinical outcome of OSCC (4-6, 15-18). Interestingly, it has been observed that irrespective of treatment regimen, tumour HPV status is a strong independent prognostic factor for survival in patients with HPV-positive OSCC (15-18). Nevertheless, smoking among patients with HPV-positive tumours significantly reduces expected survival (13, 15-17) and the relation between HPV and smoking is most likely a complex one, as briefly discussed above. It would be valuable to obtain more molecular information, and to determine if there is a survival benefit from stopping smoking during and after therapy.

In summary, it is evident that more information is necessary to guide treatment decisions for the individual OSCC patient on the basis of HPV status. However, evidence is accumulating that HPV status, overexpression of $p16^{INK4a}$, and being a never-smoker are prognostic favourable factors. Future studies including HPV, molecular biomarkers, history of smoking, cessation of smoking during therapy, as well as effects of different therapies on survival and quality of life, will be important for developing future personalized treatment.

Consequences of an HPV-induced epidemic of OSCC for prevention

As mentioned in the introduction, it is essential to recollect that we now have vaccines directed against HPV-16, which appears to be responsible for the majority of HPV-positive OSCC (4-6, 10-18). Despite the fact that it may take decades before the effects of the HPV-vaccinations on cancer incidence become obvious, their effects on the incidence of OSCC, as well as other HPV-associated tumour types merits particular consideration. One step in this direction is the recent report by Guiliano *et al*. that HPV vaccination of men can protect both against infection by HPV-6,-11,-16 and -18 and the development of anogenital lesions caused by these types (39). This is an additional argument for the inclusion of both women and men in national HPV vaccination programs.

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