



## Minireview

## Papillomaviruses in the causation of human cancers – a brief historical account

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## ABSTRACT

Approximately 35 years ago a role of human papillomaviruses (HPV) in cervical cancer has been postulated. Today it is well established that this very heterogeneous virus family harbours important human carcinogens, causing not only the vast majority of cervical, but also a substantial proportion of other anogenital and head and neck cancers. In addition, specific types have been linked to certain cutaneous cancers. In females, HPV infections on a global scale account for more than 50% of infection-linked cancers, in males for barely 5%. Vaccines against the high risk HPV types 16 and 18 represent the first preventive vaccines directly developed to protect against a major human cancer (cervical carcinoma). This review will cover some of the historical aspects of papillomavirus research; it tries briefly to analyze the present state of linking HPV to human cancers and will discuss some emerging developments.

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## Early search for an infectious etiology of cervical cancer

In a now famous paper an Italian physician, [Rigoni-Stern \(1842\)](#), analyzed death certificates of women in Verona during the period 1760–1839 and noted of a high frequency of cervical cancer in married women, widows and prostitutes, but their rare occurrence in virgins and nuns. He concluded that the development of this type of cancer should be related to sexual contacts. The rapid development of bacteriology in the second part of the 19th century resulted in early attempts to link cervical cancer to sexually transmitted infectious events, without reproducible data until the end of the 1960s. At this time first reports appeared incriminating a virus infection, Herpes simplex type 2, as candidate for cervical cancer etiology ([Rawls et al., 1968](#); [Naib et al., 1969](#); [Nahmias et al., 1970](#)). Although initially a number of confirmatory data have been published, a large scale prospective study performed in former Czechoslovakia failed to confirm these results ([Vonka et al. 1984a, 1984b](#)).

In the early 1970s studies on the possible role of human papillomaviruses (HPV) in cancers were initiated. This is discussed subsequently.

## A brief history of papillomavirus research

Skin and genital warts were well known among ancient Greek and Romans (reviewed in [Bäfverstedt, 1967](#)). Particularly genital warts were considered as the result of sexual promiscuity and thus, regarded as potentially infectious. In the end of the 19th century [Payne \(1891\)](#) reported the contagious rise of common warts. The first unequivocal demonstration of the infectious nature of human warts resulted from

cell-free transmission experiments of [Ciuffo \(1907\)](#) in Italy. This established at the same time the viral nature of the responsible agent. Within the 1920s the infectious nature of genital warts and laryngeal papillomatosis was also confirmed (see review [Syrjänen and Syrjänen, 2008](#)). Electron microscopic demonstration of viral particles was achieved in 1949 ([Strauss et al., 1949](#)).

Although important developments, demonstrating for the first time the carcinogenic potential of rabbit papillomaviruses in cotton-tail and domestic rabbits ([Rous and Beard, 1934](#); [Rous and Kidd, 1938](#); [Rous and Friedewald, 1944](#)), followed in the 1930s, in these years interest in human wart virus remained low. Nevertheless, in particular molecular studies on bovine papillomaviruses ([Heilman et al., 1980](#); [Engel et al., 1983](#)) turned out to be helpful for subsequent analyses of HPV types. In 1965 first reports appeared characterizing the double-stranded circular DNA of human papillomavirus ([Crawford, 1965](#); [Klug and Finch, 1965](#)). In a stimulating review in 1967, [Rowson and Mahy \(1967\)](#) still describe the various forms of warts and papillomas caused by *the human wart virus*. The history of epidermodysplasia verruciformis will be briefly discussed in a subsequent chapter.

In the 1970s the plurality of human papillomavirus types became apparent. An early suggestion for antigenic differences between cutaneous and genital wart papillomavirus particles originated from electron microscopic analysis of particle agglutination studies ([Almeida et al., 1969](#)). Antisera to skin wart virus reacted with both, skin and genital wart viruses, whereas antisera to genital wart virus reacted only with the genital wart virus particles ([Almeida et al. 1969](#)). This study has not been reproduced; the basis for this observation is still not understood.

A clear hint for the heterogeneity of the human papillomavirus family originated from hybridization studies with in vitro transcribed plantar papillomavirus RNA and DNA from various cutaneous, genital

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warts and cervical cancer biopsies (zur Hausen et al., 1974a,b). Although in part papillomavirus particle-positive, only a limited number of DNAs from cutaneous warts and none of the DNAs from genital warts reacted with the plantar virus RNA probe. Subsequent studies revealing the intratypic heterogeneity of plantar wart virus preparations (Gissmann and zur Hausen, 1976) and type-specific endonuclease restriction patterns of various isolates (Gissmann et al., 1977; Orth et al., 1977) clearly established the plurality of human papillomavirus types.

This review concentrates on the present role of HPV types in human cancers, by stressing some of the historical developments. It is not intended to review a large number of epidemiological or seroepidemiological data, including additional risk factors, obtained mainly during the past two decades.

### Papillomaviruses and cervical cancer

Experiments trying to establish a relationship between papillomavirus infections and cervical cancer were initiated in 1972. They were based on anecdotal reports in the medical literature of rare malignant conversion of genital warts (condylomata acuminata) into squamous cell carcinomas (reviewed in zur Hausen, 1977), resulting in the hypothesis that cervical cancer may arise from infections with the virus found in condylomata acuminata (zur Hausen 1974, 1975, 1976). The speculation appeared to be boosted by negative attempts to demonstrate herpes simplex type 2 DNA in cervical cancer biopsies (zur Hausen et al., 1974b). The isolation of novel HPV types from genital warts, HPV 6, (Gissmann and zur Hausen, 1980; de Villiers et al., 1981) and laryngeal papillomas, HPV 11, (Gissmann et al. 1982a,b) permitted direct approaches to answer this question.

In 1976 (Meisels and Fortin, 1976) and 1977 (Meisels et al., 1977), Meisels and Fortin postulated that koilocytotic cells found in cervical smears of patients with flat dysplastic lesions represent the cytopathogenic change of a papillomavirus infection. Initially these authors hypothesized that the discovery of such koilocytotic cells permitted a differentiation between the koilocyte-positive “benign” proliferations from koilocyte-negative lesions, assumed to represent “truly premalignant” cells. The demonstration of typical papillomavirus particles within koilocytotic cells by Della Torre et al. (1978) and by Hills and Lavery (1979) confirmed the HPV-mediated cytopathic effect as proposed by Meisels and Fortin.

In 1982 the first three reports on HPV sequences in human tumors were published. Early in 1982, Gissmann et al. (1982a,b) reported HPV 6 DNA in biopsies from three invasively growing giant condylomata acuminata (Buschke–Löwenstein tumors). This was confirmed in December of the same year by Zachow et al. (1982) in a series of additional Buschke–Löwenstein tumors. The result was not particularly surprising, since these invasively growing but commonly non-metastasizing tumors originate from typical genital warts. In a third report by Green et al. (1982) epidermodysplasia verruciformis HPV-related bands were demonstrated in some cervical cancer biopsies. According to the cleavage pattern they were likely to represent HPV 10. This type and other epidermodysplasia verruciformis HPV-related viruses have subsequently not been identified in cervical cancer biopsies.

Southern blot hybridizations with HPV 11 DNA permitted the detection of this DNA in one out of 24 cervical carcinoma biopsies (Gissmann et al., 1983). By using HPV 11 as probe, it was possible to isolate a novel HPV DNA directly from cervical cancer biopsies, subsequently labelled as HPV 16 (Dürst et al., 1983). Shortly thereafter, the same group isolated and partially characterized HPV 18 DNA from cervical cancer biopsies as well as from several cervical cancer derived cell lines (among them HeLa cells) (Boshart et al., 1984). Within the same year it was possible to demonstrate HPV 16 DNA in typical precursor lesions of anogenital cancers, Bowenoid papulosis (Ikenberg et al., 1983) and 1 year later in cervical intraepithelial neoplasias

(Crum et al. 1984). Without reviewing here the subsequent burst of molecular and epidemiological data within the following years, among others a few major steps deserve mentioning. Early in 1985 the selective transcription of E6 and E7 genes in cervical cancer and specific deletions occurring in the course of integration of viral DNA into host cell DNA were established (Schwarz et al., 1985). Within the same year this was confirmed by another group (Yee et al., 1985). The interaction of E6 protein with p53, resulting in degradation of this protein (Werness et al., 1990), and of E7 with pRb, blocking the function of the latter (Dyson et al., 1992), were important for the initiation and understanding of intracellular events resulting in immortalization and eventually in a transformed phenotype of the viral genome harbouring cells. Cell transformation by these viral oncogenes was initially shown for rodent cells (Yasumoto et al., 1986) and shortly thereafter also for human keratinocytes (Dürst et al., 1987; Pirisi et al., 1987). In addition, induction of tumors in transgenic animals (Lambert et al., 1993; Arbeit et al., 1993) clearly demonstrated the oncogenic potential of these genes. Global epidemiological studies identified HPV 16, 18 and a few others as major risk factors for cervical cancer (Muñoz et al., 1992; Bosch et al., 1992). The interruption of intra- and extracellularly triggered pathways as a precondition for malignant conversion (zur Hausen and Rösl, 1994; Soto et al., 2000), as well as the essential role of viral oncoprotein expression for the maintenance of the malignant phenotype (Storey et al., 1991; von Knebel Doeberitz et al., 1992, 1994), the serology of HPV infections (Stanley et al., 1994; Strickler et al., 1994), and the recent development of HPV vaccines have been covered in several reviews (e.g. zur Hausen, 1996; Schiller and Lowy, 2006; Frazer, 2007).

Today it is very well established that infection with specific types of HPV can cause cervical cancer. The intervention with vaccines permits already today the statement that essential precursor lesions of this cancer are efficiently prevented. Although more than 95% of cervical cancer biopsies contain high risk HPV genomes, this figure does not necessarily imply that all of these tumors are caused by these infections. Long-term follow-up studies of vaccinated women, particularly after achieving a broad protection against the majority of high risk HPV types, will provide a better basis for more accurate estimates. Cervical cancer, on the global scale, represents the second most frequent cancer in women. Thus, specific HPV types emerge as one of the most important infectious carcinogens in humans.

### Papillomaviruses and anogenital cancers

Verrucous carcinomas of the vulva and the penis may occasionally develop in persisting genital warts and frequently do contain HPV 6 or 11 DNA (Gissmann et al., 1982a,b; Zachow et al., 1982; Boshart and zur Hausen, 1986). Soon after the discovery of HPV 16 and 18 in cervical cancer biopsies, the same as well as additional HPV types were also found in other anogenital cancers. Early studies revealed already that Bowenoid papulosis of vulva and penis, as precursor lesions of cancers at external genital sites, were frequently positive for HPV 16 DNA (Ikenberg et al. 1983). It is presently well established that only approximately 50% of vulvar squamous cell carcinomas are HPV-positive, mainly containing HPV 16 DNA (Madsen et al., 2008). Similarly, only 30–50% of penile carcinomas contain HPV DNA (Rubin et al., 2001; Bezerra et al., 2001; Lont et al., 2006). In general, HPV-positive vulva and penile carcinomas seem to have a better prognosis in comparison with the negative tumors (Lont et al., 2006). Since many of the epidemiological risk factors for penile and vulva cancers resemble those established for cervical cancer (Hildesheim et al., 1997; Dillner et al., 2000; Rubin et al., 2001; Madsen et al., 2008), causal events leading to HPV-negative tumors at these sites represent an important open question. Different infectious causes of these cancers remain an interesting speculation.

In contrast to penile and vulva cancers, carcinomas of the vagina are in the range 60–90% HPV positive (IARC, 1995; Madsen et al.,

2008). The same seems to be true for anal and perianal cancers (Frisch et al., 1999). The global incidence of anal and perianal cancers is relatively low, ranging between 0.1 to 2.8 cases per 100,000 (WHOCT, 2000). Sexual habits, men having sex with men (MSM), lead to a dramatically increased risk to an estimated incidence rate of 35 per 100,000 that may go up to 70 per 100,000 among HIV-seropositive MSM (Daling et al., 1987). Early demonstration of HPV 16 in anal carcinomas dates back to 1986 (Scheurlen et al. 1986; Hill and Coghill, 1986). In the same year a report described the frequent occurrence of anal intraepithelial dysplastic lesions in homosexual men (Nash et al., 1986).

### Papillomaviruses and head and neck cancers

A number of early reports discussed the malignant conversion of recurrent laryngeal papillomas into squamous cell carcinomas, dating back in part to the 1940s and 1950s (reviewed in zur Hausen 1977). Interestingly, an early report also claimed a 5–6 fold increased risk of women with cervical cancer for the subsequent development of oral cancer (Newell et al., 1975). Syrjänen and colleagues (1983) reported positive immunoperoxidase staining with anti-HPV serum in oral focal epithelial hyperplasia and oral squamous cell papillomas. The demonstration of papillomavirus antigens in premalignant lesions of the oropharynx provided first hints for a possible role of papillomavirus infections in oral squamous cell carcinomas (Syrjänen et al., 1983). The first unequivocal reports of specific HPV types in tongue and other oropharyngeal carcinomas appeared in 1985 (Löning et al., 1985; de Villiers et al., 1985). Three of 13 carcinomas tested contained HPV 16 DNA, one was HPV 11 positive, and one contained HPV 27 DNA (initially labelled as variant HPV 2). In subsequent years a large number of studies confirmed these data, although the rate of positivity varied commonly between 25 and 60% (Weinberger et al., 2006; Psyrri and DiMaio, 2008; Gillison et al., 2008). In a more detailed analysis Weinberger et al. (2006) demonstrated that out of 61% of HPV 16-positive oropharyngeal carcinomas 23% revealed a similar expression pattern of p16 as observed in cervical cancers. This raises the question whether some of the other HPV-positive tumors may have originated from the contamination of the tumor tissue with HPV 16 from virus-production at other non-malignant oropharyngeal sites of the same patient. This point requires further investigations. A conservative estimate of 25–30% of oropharyngeal cancers probably caused by high risk HPVs may be closer to reality.

A few aspects are interesting to note: according to the SEER statistics, in the USA the annual incidence of base-tongue and tonsil cancers among white men and women aged 20–44 years increased between 1973 and 2001 by 2.1 and 3.9%, respectively (Shiboski et al., 2005). Sexual practices seem to be partially responsible for the acquisition of anogenital HPV infections in the oral cavity (Schwartz et al., 1998; D'Souza et al., 2007). Patients with anogenital cancers had a 4.3-fold increased risk of tonsillar cancers (Frisch and Biggar, 1999). In tonsillar cancer a particularly high rate of HPV positivity had been noted, accompanied by E6 and E7 gene expression (Snijders et al., 1992). A better prognosis has been reported for HPV positive in comparison to HPV-negative oral squamous cell carcinomas HPV-linked oropharyngeal cancers, with a 60–80% reduction in risk of death (Psyrri and DiMaio, 2008).

In summary, approximately on quarter to one third of oropharyngeal cancers are probably caused by anogenital high risk HPV infections.

A low number of verrucous carcinomas of the larynx may develop after prolonged episodes of recurrent papillomatosis (Abramson et al., 1985; Brandsma et al., 1986), occasionally also within the bronchial tract (reviewed in zur Hausen 1974). They contain HPV 16, HPV 11 or HPV 6 DNA (reviewed in Steinberg, 1990). First reports on HPV types in spontaneously arising laryngeal cancers (HPV 16 and HPV 30) appeared in 1986 (Kahn et al., 1986; Scheurlen et al., 1986). It seems

today that the vast majority of laryngeal carcinomas are negative for established HPV types and that the percentage of positive cancers at this site does not exceed 5%.

### Papillomaviruses and skin cancers

Although not recognized at that time, the history of papillomavirus infections and cancer of the skin dates back to the year 1922, when Lewandowsky and Lutz (1922) described a rare hereditary condition, epidermodysplasia verruciformis. Particularly at light-exposed sites, large areas of the skin are covered by flat papillomatous lesions, some of which may convert into squamous cell carcinomas after one or two decades of persistence. Within the 1960s typical papillomavirus particles have been detected electron microscopically within the papillomatous lesions, but not in malignant tissue (Ruiter and Van Mullem, 1970; Yabe et al., 1969). Even prior to their demonstration, the infectious nature of these lesions was shown in auto- and hetero-inoculation experiments, where cell-free extracts resulted in the induction of flat warts (Lutz, 1946; Jablonska and Millewski, 1957; Jablonska and Formas, 1959).

In 1978, Orth et al. (1978) reported the presence of specific HPV types in malignant lesions of epidermodysplasia patients HPV 5 and to a lesser extent a related virus, HPV 8, and HPVs 12, 14, 17, or 20 are found in malignant lesions (reviewed in Orth, 1986). Experimental data support a role of these viruses in the conversion of skin cells towards malignant growth. This is in particular supported by the development of skin tumors in mice transgenic for early genes of HPV 8 (Schaper et al., 2005) and HPV 20 (Michel et al., 2006). Relatively little is known on transcription and expression of viral early genes within human malignant lesions. No cell cultures have yet been established from Epidermodysplasia verruciformis (EV) lesions. EV represents a rare, autosomal recessive dermatosis, commonly accompanied by inactivating mutations in the related *EVER1/TMC6* and *EVER2/TMC8* genes (reviewed in Orth, 2008). Thus, in spite of the regular presence of specific HPV types in the malignant lesions of EV patients and a larger number of experimental data on HPV 5, 8 and 20 gene functions, too few studies exist analyzing directly the malignant tumors. The interaction of these viruses with UV light, studied for HPV 20 (Ruhland and de Villiers, 2001) should require further attention.

Warts and squamous cell carcinomas (SSC) of the skin arise as a frequent complication in organ transplant recipients (reviewed in Bouwes Bavinck et al., 2001). Although studied for more than 2 decades, the role of HPV types in SSC induction remains unclear. This is in part due to the diverse group of HPV types found in these lesions, but also in normal skin, and the presence of multiple types within the same biopsy and or skin abrasion. An initial report described the presence of HPV 5 DNA in skin cancers of an immunosuppressed renal allograft recipient (Lutzner et al., 1983), followed by two reports in 1994 of a high rate of HPV positivity (40–56%) in SSCs of immunosuppressed patients (Stark et al., 1994; Shamanin et al., 1994). In the following years the plurality of HPV types in these cancers was confirmed, the low copy number of far less than 1 genome copy per tumor cell and the high rate of positive data from normal skin materials and plucked hair render an interpretation of these data difficult. A possible indirect function of the infecting HPV types in skin carcinogenesis may originate from observations demonstrating the inhibition of apoptosis by E6 of some cutaneous HPV types (Jackson et al., 2000) and the degradation of E6 of HPV 20 by p53 (Fei and de Villiers, 2008). The latter observation contrasts data obtained for high risk anogenital HPVs and could provide a reasonable explanation for syncarcinogenic effects of HPV infections and sunlight exposure. p53 mutations induced by the UV part of sunlight (see review de Gruijl and Rebel, 2008) would permit an unimpaired expression of viral oncoproteins. This, however, requires further investigations.

Thus, the role of HPV types in squamous cell carcinomas of the skin is not yet resolved.

### Other cancers linked to papillomavirus infections

A number of individual reports claim mainly the presence of anogenital human papillomavirus types in cancers of the esophagus, prostate, bladder, breast, lung and other organ sites (see review IARC, Human Papillomaviruses, Volume 90, 2008). With the exception of nail bed cancers, none of these reports demonstrated a consistent association of these viruses with cancer of the respective sites. Although it is likely that high risk HPV types may occasionally cause cancers in atypical locations, the inconsistency of these reports does not warrant an intensive discussion in this review.

It should be noted, however, that most present searches for novel HPV rest on the use of established consensus primers in polymerase chain reactions, permitting to some degree the detection of only partially homologous sequences. Theoretically, HPV types not discovered by these primers and very distantly related to known type of this virus group would in all likelihood escape detection. An interesting example of a very novel virus type, apparently representing a kind of chimera between late papillomavirus sequences and an early region organization of a typical polyoma-type virus has recently isolated from skin lesions of an Australian marsupial, the bandicoot (Woolford et al., 2007; Bennett et al., 2008). Its sequence relationship to known members of either of the two virus families is low and would not permit their discovery by using consensus primers presently developed for known HPV or polyoma-type viruses. The identification of such agents would be particularly difficult if they originate from another species, are for instance replication-defective in human cells, but still could express early functions that in a specifically "conditioned" cell may lead to malignant transformation (zur Hausen, 2008).

The recent isolation of a novel polyoma-type virus from Merkel cell carcinomas (Feng et al., 2008) in the future may draw more attention to this structurally related virus family. There are still a large number of skin cancers, vulva and penile cancers negative for identified HPV types. The incidence of all of these cancers is substantially increased under long-term immunosuppression (Vajdic et al., 2006) which may hint to a possible infectious etiology of these lesions.

### Conclusions

Although the history of papillomavirus research reaches far back to the beginning of the last century, only advances made in the 1970s permitted an analysis of the plurality of HPV types and the establishment of a role of specific types in cervical cancer. Additional evidence today stresses a role of the same types in anal cancer, in up to 50% of other anogenital cancers, and in 25 to 30% of head and neck cancers. The role of HPV in squamous cell carcinomas of the skin is less well established, even though a rare hereditary condition with malignant conversion of papillomatous lesions at sun-exposed sites, epidermodysplasia verruciformis, has been linked to infections with additional HPV types. Thus, members of the papillomavirus family turn out to be particularly important human carcinogens.

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