# INVITED SPEAKERS

# THE HISTORY OF PAPILLOMAVIRUS RESEARCH

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## INTRODUCTION

The present-day understanding of papillomavirus (HPV) research and clinical practice is a result of a long history of hard work consisting of important contributions of a countless number of past and present scientists. PV research has an interesting dual type of the past history; 1. basic virological data emerging from the animal experiments during the first half of last century, and 2. an increasing awareness of these viruses as a significant cause of human diseases emerging from the late 1970's.

Here we present milestones of the of HPV research in chronological list (Tables 1, 2 and 3). Like always, this type of listing represents the personal preferences of the authors, and the missing of someone's name in the list by no means signify the lack of importance of his/her work. The major focus of the listing is in the studies on HPVs, and the historical data on animal PVs are listed only when having immediate implications in understanding of the human disease. During the past several decades, several excellent reviews have been written on the history of papillomavirus research (1–10).

## OBSERVATIONS MADE BEFORE 1970

Genital warts were well known to physicians of the ancient world, at least from the time of Hippocrates (460–377 B.C.). Since those days, the term condyloma (a word of Greek origin) has been used for genital warts meaning "a round swelling around the anus". At the end of the 19th century, the genital wart has been called condyloma accuminatum, "a pointed condyloma" (8). Since Antiquity, condylomata accuminata have been linked with sexual behavior, being particularly common in the anal region of homosexual males.

Also the skin warts have been known since the Roman Era. At the beginning of the 1st century A.D., Celsus described three morphologically distinct types of cutaneous warts. These included 1. acrochordon, which was encountered exclusively in children and frequently underwent spontaneous resolution, 2. thymion, which was a papillomatous and highly vascular lesion, and 3. myrmecia, currently known as plantar warts.

In the virological point of view, nothing dramatic took place until the early 20th century. However, an important report from the 19th century deserves to be cited here. This document was published by an Italian physician, Rigoni-Stern in 1842, who analysed the certificates of death due to cancer in Verona during the period 1760–1839 (11). He found that deaths due to cancer of the uterus were very rare among virgins and nuns, as contrasted to married women and widows, among whom the disease was quite common in those days. Thus, he was the fist to link cervical cancer with the sexual contacts.

It took another 50 years, however, until the infectious nature of cutaneous warts was reported by Payne (1891) and Jadassohn (1896) (12,13). This contagious mode of transmission was confirmed also for genital condylomas some 10 years later, when Heidingsfield described a prostitute, who had acquired condyloma lesions in her tongue as a result of oral sex (14). Only a few years later, the viral etiology of these lesions was demonstrated by Ciuffo in 1907, who used a cell filtrate of a common wart to transfer the infection (15). Human wart virus was later associated with genital (16) and laryngeal (17) warts. Ullman also claimed to have produced a papilloma of the vagina of a bitch upon inoculation with extract of laryngeal papilloma, but Findlay (18) was unable to confirm this finding. It took some 40 more years, however, before HPV was isolated as a microcrystalline form by Strauss et al. (19). During the subsequent twenty years, it was believed that all different manifestations of warts, both cutaneous and genital, were caused by a single type of the virus known as the human wart virus.

During this period of slow progress with understanding the clinical disease, some important breakthroughs were made in experimental animal studies. Shope and Hurst in 1933 were the first to describe a skin papilloma in wild, North American cottontail rabbits (20). These cutaneous papillomas could be

***Table 1.*** *Key discoveries signifying milestones in HPV research before 1970*

| Year | Author | Discovery |
|---|---|---|
| 460-377 B.C. | Hippocrates | The name condyloma endowed to genital warts |
| 25 A.D. | Celsus | Three types of skin warts: acrochordon, thymion, myrmecia |
| 1842 | Rigoni-Stern | "Venereal" nature of cervical cancer: rare in virgins and nuns |
| 1891 | Payne | Infectious nature of common warts |
| 1901 | Heidingsfield | Oral condylomas: a sign of illicit sex |
| 1907 | Ciuffo | Demonstration of the viral nature of skin warts |
| 1923 | Ullman | Demonstration of the viral nature of laryngeal papillmoas |
| 1933 | Shope & Hurst | Isolation and characterization of cotton-tail rabbit papillomavirus (CRPV) |
| 1935 | Rous & Beard | Development of rabbit skin carcinomas due to synergistic actions etween CRPV and chemical carcinogens |
| 1938 | Kidd & Rous | |
| 1944 | Friedewald & Rous | |
| 1949 | Parson and Kidd | Natural history of rabbits oral papillomas |
| 1949 | Ayre & Ayre | Halo cells in cervical carcinogenesis |
| 1949 | Strauss et al. | HPV particles in skin warts on EM |
| 1950 | Syverton et al. | Papilloma-to-carcinoma sequence in cottontail rabbits |
| 1951 | Olson et al. | Equine sarcoids caused by an agent of bovine papilloma |
| 1954 | Barrett & Silbar | Genital warts shown to be an STD |
| 1956 | Koss & Durfee | The concept of koilocytosis in PAP smears and cervical biopsies |
| 1957 | Jablonska et al. | Revival of epidermodysplasia verruciformis as a viral disease |
| 1961 | Ito & Evans | Induction of papillomas in domestic rabbits by CRPV |
| 1965 | Crawford; Klug & Finch | Structural and molecular characterization (mw) of papillomavirus DNA exctracted from skin warts |
| 1962 | Melnick | Human Papova virus group |
| 1967 | Rowson & Mahy | |
| 1968 | Dunn & Ogilvie | EM demonstration of HPV particles in genital warts |
| 1969 | Almeida et al. | |

***Table 2.*** *Key discoveries signifying milestones in HPV research during the 1970's*

| Year | Author | Discovery |
|---|---|---|
| 1972-75 | Pyrhönen et al. | Preliminary studies on antibody response to cutaneous and genital warts |
| 1974 | zur Hausen | HPV types differ in plantar and genital warts |
| 1975-76 | zur Hausen | Hypothesis presented on HPV as a potential cause of cervical cancer |
| 1976 | Gissmann & zur Hausen | Diversity of papillomavirus types |
| 1977 | Gissmann et al. | |
| 1977 | Orth et al. | |
| 1976 | Meisels et al. | Description of koilocytotic atypia as a sign of HPV in flat lesions of the uterine cervix |
| 1977 | Purola & Savia | |
| 1978 | Pyrhönen et al. | Wart virus antibodies detected in dogs, pigs and cattle |
| 1978 | Della Torre et al. | Demonstration of HPV particles in dysplastic lesions of the cervix on EM |
| 1979 | Hills & Laverty | |
| 1978 | Orth et al. | Specific HPV types in EV-derived skin carcinomas |
| 1978-80 | Quick et al. | Classical works on laryngeal papillomas, their atypia and association with HPVs |
| 1979 | Syrjänen, K. | Koilocytotic cells in invasive cervical cancer |
| 1979 | Law et al. | Gene cloning and hybridization under non-stringent conditions in HPV research |
| 1979 | Syrjänen, K. | HPV-suggestive changes in bronchial squamous cell cancer |
| 1979 | zur Hausen & Coggin | System for HPV classification |

induced in both wild cottontail rabbits and in domestic rabbits using either purified or filtered homogenates of the tumours. In subsequent experiments, these authors showed that the disease was not transmitted among the domestic rabbits but readily from the wild rabbits to domestic rabbits (Cottontail rabbit papillomavirus, CRPV) (20).

These primary observations were followed by a period of intense experimentation with this new papilloma model, conducted particularly by Peyton Rous and his colleagues between 1935–1944 (21–23). They convincingly demonstrated the importance of synergistic actions between CRPV and chemical carcinogens in the malignant transformation of the benign cutaneous papil-

***Table 3.*** *Key discoveries signifying milestones in HPV research since 1980 up to now*

| Year | Author | Discovery |
|---|---|---|
| 1980 | Gissmann & zur Hausen | Characterization of HPV-6 from a condyloma accuminatum lesion |
| 1981 | de Villiers | Cloning of HPV 6 in bacterial vectors |
| 1982 | Gissmann et al. | Cloning and characterization of HPV-11 from a laryngeal papilloma |
| 1981 | Syrjänen et al. | The first prospective follow-up study for cervical HPV infections initiated |
| 1982 | Syrjänen et al. | HPV involvement in squamous cell papilloma and cancer of the oesophagus |
| 1983 | Syrjänen et al. | HPV in inverted papillomas of the nasal cavity/paranasal sinuses |
| 1983 | Dürst et al. | Characterization of HPV-16 in cervical cancer biopsies |
| 1982 | Jenson et al. | HPV involvement in oral squamous cell lesions (benign & malignant) |
| 1983 | Syrjänen, S. et al. | |
| 1984 | Boshart et al. | Characterization of HPV-18 DNA in cervical cancer biopsies |
| 1985 | Kreider | Condyloma-type lesions induced by HPV-11 keratinocytes grafted in nude mice |
| 1985 | Beckman et al.<br>Gupta et al. | *In situ* hybridization in cervical biopsies |
| 1986 | Lörincz et al. | Characterization of HPV-31 |
| 1986 | Beaudenon et al. | Characterization of HPV-33 |
| 1986 | Yasumoto et al. | Malignant transformation of NIH3T3 cells by HPV-16 DNA |
| 1987 | Pirisi et al.<br>Dürst et al. | Immortalization of human keratinocytes by HPV-16 DNA |
| 1988 | Laimins et al. | Organotypic raft culture as an *in vitro* model in HPV research |
| 1989 | Dyson et al. | HPV E7 binding with Rb protein |
| 1990 | Werness et al. | HPV E6 binding with p53 protein |
| 1992 | Kirnbauer et al. | Production of virus-like particles by L1 and L2 self assembly |
| 2006 | Merck; GSK | FDA licensed a quadrivalent HPV vaccine |

lomas. Experiments on these lines were continued by Syverton, who accurately described the papilloma-to-carcinoma sequence in both the domestic and cottontail rabbits (24). In 1943, Parson and Kidd published their milestone study where they showed that oral papillomatosis of rabbits is a viral disease and the oral papillomavirus was distinct from the skin papilloma virus (25). They also found that virus may somehow pass from the mothers to the young during the period of suckling and this in turn brought up the possibility that the virus might lie latent in the mouth of normal rabbits until favorable circumstances (injury or irritation) (25). The data of that paper are still actual and no similar natural history studies on oral papillomas in humans exist even today.

Since the early 1950's, increasing attention was paid to papillomavirus-induced tumours in domestic animals as well. Equine sarcoids are the most frequent spontaneously occurring tumours in horses. These connective tissue tumours usually do not metastasize but they may be locally invasive and capable of recurring even after a radical surgical excision. The etiological role of Bovine papillomavirus (BPV) was first suggested by the transmission experiments of Olson and Cook (26), the agent having been confirmed as BPV1 and BPV2 by other workers later.

Despite the persistent ancient belief that genital warts are linked with sexual habits, it was not until 1954, when the sexual transmission of genital condylomata accuminata was firmly established (27). These authors examined young American soldiers returning from the Korean war. After having had sexual intercourse with the local prostitutes in Korea, they transmitted the disease to their sexual partners in the U.S., who usually developed genital condylomas after 4 to 6 weeks of incubation period. Since its original description, the sexual mode of HPV transmission has been firmly established.

The 1950's also witnessed the appearance of two important papers giving cytological descriptions of genital HPV infections. In 1949, Ayre et al. described the morphology of halo cells in PAP smears and cervical biopsies, calling them as a precancer cell complex (28). One of their patients subsequently developed a carcinoma in situ (CIS) lesion, and the authors renamed this cytological abnormality as a "nearocarcinoma" in 1951. While studying the cytological smears taken during a pilot screening program, Koss and Durfee could confirm Ayre's discovery. They published their classical paper in 1956, giving this cellular abnormality a new name: koilocytotic atypia (29). The intriguing history of these early steps in describing the cytopathology of cervical cancer precursors was thoroughly revisited by Koss himself (30). The author admits, however, that the viral etiology of the koilocytotic atypia was not suspected in 1956, although the wart-like epithelial changes pointed to that direction (30). This same decade also saw the revival of interest in yet another clinical entity described in 1922 by Lewandowski and Lutz (31) as epidermodysplasia verruciformis (EV), when Jablonska published her classical paper on this subject in 1957 (32).

DNA or viral particles extracted from cottontail rabbit papillomas were shown to induce papillomas by Ito and Evans in 1961 (33). It still took a number of years until the genome of this CRPV was fully characterized. With the developing research techniques in 1960's, also human wart virus attracted new interest among virologists. In 1965, two groups (34, 35) characterized the structure and molecular weight of papillomavirus DNA extracted from skin warts. Finally, towards the end of 1960's, viral particles were finally demonstrated by electron microscopy also in genital warts (36, 37). Because of the fact that this virus was morphologically identical with the particles found in skin warts, it was believed

that both genital and skin warts are due to a single wart virus. By that time, this virus was included as a member in Human Papova virus family (38, 39).

## DISCOVERIES MADE IN THE 1970'S

The 1970's witnessed a number of major breakthroughs in many aspects of HPV research, representing both clinical discoveries and those made by basic scientists, the key discoveries being summarized in Table 2. The onset of the 1970's was heralded by the emerging data on serological response to human wart viruses. The pioneering observations of a Finnish virologist, Pyrhönen dating back to the early 1970's have received far too little attention (40–43). The authors detected measurable antibodies against pooled, homogenized wart tissue in 92% of the women and 75% of the males aging between 15–19 years. Important was also their observation on a roughly inverse correlation between the number and duration of skin warts and the antibody levels. While studying the patients with SLE, who showed HPV antibodies less frequently than their immunocompetent counterparts, the authors concluded that this lack of humoral response to HPV might explain the increased frequency of warts in SLE patients. They also speculated the antigenic relatedness between the HPVs in the genital and skin warts, as suggested by the less frequent occurrence of HPV antibodies in genital wart patients. While detecting measurable HPV antibodies in students reporting never having had visible wart lesions, the authors made the assumption that subclinical HPV infections must be quite common (43). They also concluded that there must be more than one HPV type.

HPV research of today owes much to one of the pioneers in the field, Dr. Harald zur Hausen, who turned his interest on HPV in the early 1970's. In the first of his classical series of 4 works from 1974–1976, attempting to detect virus specific DNA in human tumors, he completed nucleic acid hybridizations with complementary RNA of human wart virus (44). It became evident that different viruses are responsible for cutaneous common warts and genital warts. During the same period, he formulated his hypothesis on HPV as an etiological agent of cervical cancer (45, 46). In a series of studies from 1976–77, zur Hausen and his coworkers as well as Gerard Orth's group in Paris, established the plurality of HPVs, by disclosing the first 4 HPV types in cutaneous warts, numbered as HPV 1–4 (47–49). Also serological evidence was soon provided to support this plurality, because there seemed to be no link between HPV 1–4 and the HPV type (to be characterized) in condylomata accuminata, laryngeal papillomas or any of the malignant tumors tested (50).

From the clinical point of view, a major breakthrough was provided by two reports appearing in two subsequent issues (Nov-Dec & Jan-Feb) of Acta Cytologica in 1976 and 1977 (51, 52) by describing koilocytotic cells in cervical Papanicolaou (PAP) smears derived from flat epithelial lesions, frequently associated with cervical precancer lesions. In addition to characteristic koilocytes, these authors accurately described two other types of cells regularly exfoliating from genital condylomas; dyskeratotic superficial cells, and "condylomatous" intermediate cells (51, 52). It was soon realised that by observing the cytopathic effects of a virus on light microscopy, one could probably "see" the evidence for an etiological agent of cervical cancer and its precursor lesions.

Della Torre and her colleagues as well as Hills & Laverty were the first to detect viral particles within the nuclei of koilocytes (53, 54) which were identical to those described in condylomata accuminata (36, 37), and in skin warts (19). This confirmed that also these flat and endophytic epithelial lesions of the genital tract are manifestations of an HPV infection, although distinct from the classical condyloma accuminatum in morphological appearance. This circuit was closed in 1979, when a case of invasive cervical squamous cell carcinoma was described with abundant koilocytes (55).

In 1978, two new HPV types associated with EV lesions were discovered by Orth and his group paving the way to subsequent detection of a large number of so called "EV-specific" HPV types recognized today (56).

In parallel with the incited interest in HPV lesions of the genital tract and skin, the suspected HPV origin of two additional lesions was confirmed; first with the juvenile-onset laryngeal papillomas and later with the adult-onset papillomas. Quick and coworkers described epithelial atypia in these lesions, with possible implications in their known risk for malignant transformation (57–59). Soon, conclusive clinical and virological evidence on similarities between genital condylomas and laryngeal papillomas was provided (59). Within the next two years, HPV involvement in laryngeal and esophageal squamous cell carcinomas was suggested by us, based on morphological features and detection of HPV antigens by immunohistochemistry (IHC) (60–62).

Before the introduction of DNA technology into general use, morphology, TEM and IHC were the means to provide evidence for the possible HPV involvement in genital and extra-genital squamous cell lesions. With these methods, we described koilocytotic cells and other morphological evidence (confirmed by IHC) suggestive of HPV involvement in bronchial squamous cell carcinomas (63) paving the way to a novel thinking about the potential HPV etiology of human squamous cell carcinomas at mucosal sites other than the genital tract, larynx or skin.

The decade was closed by an introduction of a unanimously agreed classification of PVs, based on the decisions of the first Workshop on papillomaviruses (64). This first classification was based exclusively on the sequence homology between the different PV isolates. Accordingly, viral DNA isolates are first classified according to their host species (HPV, BPV, CRPV, etc.) (see ref. 65), and within one species, according to their sequence homology: if there is less than 50% cross-hybridization with the known PV types when tested by re-association kinetics in the liquid phase, the viral DNA was assigned a new type. This classification prevailed until the early 1990's, when revised and finally PVs were classified as a taxonomic family of their own in 2004 (66).

## INCREASING SPEED OF PROGRESS SINCE 1980

The 1980's witnessed a remarkable and dramatic progress in all areas of PV research mostly because of the development of molecular cloning and related techniques. This speed is well illustrated by the rapid discovery, cloning and characterization of an ever growing number of different HPV types, for which there is no end visible today. This development was started in 1980 by

Gissmann and zur Hausen, who isolated and characterized a new virus, which proved to be the etiological agent of classical genital warts and was designated as HPV-6 (67). Characterization of the first of these genital HPV types led to isolation of its closest relative from laryngeal papilloma receiving the label HPV-11 (68). At that time, all attempts to detect homologous DNA in laryngeal squamous cell carcinomas failed, however (68).

One of the absolute highlights of the early 1980's was the isolation and characterization of a new HPV type from cervical cancer, which subsequently has proved to be the single most important HPV type of them all, namely HPV-16, by Dürst and his colleagues in 1983 (69). While tested in a series of biopsies from cervical, vulvar, and penile cancer, >60% of cervical cancer samples were found to hybridize with HPV-16 DNA, the corresponding figures for vulvar and penile cancer being 28.6% and 25%, respectively. In contrast, practically none of the benign condyloma lesions were shown to contain HPV-16 DNA, which led the authors to suggest that HPV-16 is an HPV type characteristic to malignant squamous cell lesions of the genital tract.

In 1984, HPV-18 was isolated and characterized from cervical carcinoma (70). Importantly, HPV-18 DNA was also found in several cell lines derived from cervical cancer, including HeLa, KB and C4-1 lines (70). In 1986 Lörincz and coworkers isolated HPV-31 (71), and HPV type 33 was molecularly cloned and characterized by the French group in the same year (72).

Although it may sound egocentric to include a number of one's own contributions in the list like this, the authors feel it justified to mention October 1981, the starting point of which probably is the most significant contribution of us to HPV research; the first prospective follow-up (cohort) study of women with HPV infection in the lower genital tract. By 1985, we had established that the natural history of cervical HPV lesions was identical with that of classical CIN lesions. In the same year, the inherent potential of HPV-16 and HPV-18 lesions to progress to invasive cancer was firmly established (73). Also the first epidemiological study on risk factors of HPV appeared at the same time, confirming the early onset of sexual activity, number of sexual partners, contraception mode as well as smoking among the key risk factors of HPV infections (74).

At the same time the authors did not loose their interest in exploring the potential HPV etiology of squamous cell tumours at other anatomical sites. This search led to description of such evidence in two distinct entities, squamous cell papilloma and carcinoma of the oesophagus in 1982 (75), followed a year afterwards by yet another squamous cell lesion, inverted papilloma of the nasal cavity/paranasal sinuses (76). The latter represent relatively rare tumours but have clinical importance due to their frequent tendency for recurrence (known before any evidence for HPV), and a small but definite risk for malignant transformation.

The same period witnessed extension of HPV research into yet another group of squamous cell lesions, subsequently gained a substantial clinical importance, i.e., the first evidence on HPV involvement in benign (77), and malignant (78) squamous cell tumours of the oral mucosa. The authors well remember that at that time, it was extremely difficult to get the reports on such a new idea (a potential etiological agent of oral cancer) accepted in any journals of oral medicine, which resulted in a marked delay in the publication of these studies (78,79). It was readily apparent that signs of HPV were found in oral condylomas, oral common warts and focal epithelial hyperplasia (FEH) lesions (78, 79). Evidence was also provided, for the first time, that HPV may be involved in oral squamous cell carcinomas as well.

The mid 1980's also brought significant achievements in the methodological development of HPV research. Undoubtedly, two events on these lines deserve to be listed here: 1. the description of so called Kreider model, and 2. development of different hybridization methods. Kreider et al. in 1985 made experiments, where they succeeded in inducing epithelial changes consistent with condyloma accuminatum in epithelial cells derived from normal human uterine cervix after exposure to HPV-11 from a condyloma accuminatum lesion (80). This was the first demonstration of a morphological transformation of human tissues with an HPV under controlled, experimental conditions. The only disadvantage of the model is the failure of the grafted epithelium to support the replication of the oncogenic HPV types, e.g. HPV-16 and 18.

In parallel with the advances made in technology, also the basic understanding of the mechanisms how PVs induce malignant transformation did substantially increase through the innovative experiments, meritorious enough to be cited here. In 1986, Yasumoto and his coworkers described the transformation of a rodent cell line (NIH3T3) by HPV-16 (81). Using a recombinant HPV-16 DNA (pSHPV16d), which contains a head-to-tail dimer of the full-length HPV-16 genome, they could induce morphologic transformation, and indeed, the transformed cells proved to be tumourigenic in nude mice. Subsequently, the transformation of NIH3T3 cells has provided a useful model for analyzing the functions of HPV-16.

At least equally important are the two reports from 1987 (82, 83), where human keratinocytes and fibroblasts isolated from foreskin were transformed by transfection with recombinant HPV-16 DNA. The transformed cells exhibited an extended (fibroblasts) or indefinite (keratinocytes) life-span compared with that of normal controls. Such immortalized cell lines represent an unique system to study the interaction of HPV with its natural target cell *in vitro* (83).

Yet another technical innovation, albeit not an original development, was made in the late 1980's, when the organotypic raft culture was adopted in HPV research in Dr. Laimins' laboratory (84). Using a cell culture system for keratinocytes which allows stratification and production of differentiation-specific keratins, the authors examined the effects of HPV-16 on the differentiation capabilities of human epithelial cells. The histological abnormalities induced by HPV-16 closely resembled those seen in CIN lesions (84).

While approaching the 1990's, two key discoveries in the basic HPV research absolutely deserve their place in the list, because significantly contributing to our understanding about the basic mechanisms of virus-host cell interactions. Prompted by the previous observations that the Rb1 (retinoblastoma susceptibility) gene product, p105-RB, forms stable complexes with the oncoproteins of the adenovirus E1A proteins and the SV40 large T antigen, Dyson and coworkers in 1989 demonstrated that the E7 oncoprotein of HPV-16 can form similar complexes with p105-Rb (85). While a similar mechanisms in transformation was used by these three DNA viruses, the findings strongly implicated Rb-binding as a possible step in HPV-associated carcinogenesis (85).

This discovery was followed by a logical approach to explore, whether another tumour suppressor gene, p53, and its protein

product will bind to yet another oncoproteins of HPVs. Accordingly, Werness et al. in the same year (86), showed that the E6 protein of HPV-16 is capable of binding to the cellular p53 protein. Because of the fact that the wild-type p53 protein also forms complexes with the SV40 large T antigen and the E1B 55-kD protein of adenovirus type 5, an analogy was established between E7-Rb and E6-p53 complexing. Undoubtedly, this was a strong evidence in favor of the concept that HPVs, adenoviruses, and SV40 utilize similar cellular pathways in their transformation (86). Since these experiments, more than twenty human proteins have been identified interacting with either HPV-16 E6 or E7 proteins.

The closer we approach the present day, the more difficult it becomes to name the key discoveries on PV research. This is because of the fact that the literature is crowded by almost weekly reports on carefully conducted experiments, penetrating piece by piece into the secrets of HPVs and their interactions with the host cell machinery. However, important as they might be, the final significance of these newly reported data remains to be fully appreciated only after a few years.

As the last account in this long chain of discoveries, the authors selected just one from the 1990's, which already by now has opened completely new visions into at least two important areas of HPV research: serology and vaccination. This of course is the description of the technique how to make virus-like particles (VLP) *in vitro*, by Kirnbauer and his associates (87). These authors succeeded in expressing the L1 major capsid proteins of BPV1 and HPV-16 in insect cells using a baculovirus vector and analyzed their conformation and immunogenicity. The L1 proteins were expressed at high levels and, surprisingly, assembled into structures that closely resembled PV virions (87). These self-assembled BPV L1 VLPs mimicked intact BPV virions, e.g. being capable of inducing neutralizing antisera in rabbits. Thus, the L1 protein seemed to have an intrinsic capacity to assemble into empty capsid-like structures with immunogenicity similar to that of infectious virions. This novel L1 VLP preparation was immediately recognized as a potential candidate for serological tests to measure antibodies to conformational virion epitopes, as well as for a vaccine to prevent HPV infections (87). Subsequent studies on these lines have resulted in the development of the first-generation prophylactic vaccines against HPV-6, -11, -16, -18 (Gardasil®, Merck) or against HPV-16 and HPV-18 (Cervarix®, GSK).

With the current understanding of the role of HPVs in human carcinogenesis and viewing the WHO incidence- and mortality statistics of 15 major malignancies worldwide, it can be estimated that HPVs might be involved in the development of at least 5% of all human malignancies. This fact alone should justify the distribution of updated information on HPVs as a cause of major human pathology not only to gynecologists and dermato-venereologists, but to specialists in many other fields of medicine as well.

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