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Review article

Human papillomaviruses in urological malignancies: A critical assessment

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Abstract

Objectives: Infection with human papillomaviruses (HPVs) is intimately associated with anogenital tract malignancies including cervical and vulvar cancer, a subset of oropharyngeal cancers and certain types of skin cancer. A number of urological tumors have likewise been suggested to be associated with high-risk HPV infection; however, many studies are hampered by a limited number of detection methods. The goal of this review article is to define a set of key criteria when implicating a virus in a human cancer and to apply these criteria to HPV infection in urological cancers.

Materials and methods: We performed a survey of the literature to corroborate the evidence to support a causal relationship between HPV infection and major urological malignancies.

Results: A number of previous reports have implicated HPVs in urological malignancies including penile, prostate, and bladder cancer. Most reports, however, rely only on a limited number of detection methods and frequently use contamination-prone polymerase chain reaction based methods. To firmly establish a link between a viral infection and a human malignancy, it is paramount that an array of techniques is employed and that the virus is ultimately traced by either direct visualization or, in the case of viral genome that has integrated into the host genome, detection of viral genes and gene products as well as functional cellular perturbations. In any case, seroepidemiological studies are likewise crucial to support the evidence. Such evidence for a role of HPV in urological malignancies based on currently available techniques is only present for penile squamous cell carcinomas.

Conclusions: An increasing number of immunocompromised patients as well as novel developments in patient care may change the spectrum of HPV-associated neoplasms. This is examplified by results demonstrating a role of HPVs in rare urothelial carcinomas with squamous differentiation in patients with neurogenic bladder. Hence, it is important to keep HPV infection in mind when confronted with unusual disease manifestations of the urogenital tract.

Keywords: Human papillomavirus; HPV; Urological cancers

1. Introduction

Human papillomaviruses (HPVs) are one of the most common sexually transmitted viruses worldwide [1]. Most sexually active individuals become infected with HPVs during their lifetime. For the majority of the population, HPV infection is asymptomatic, self-limiting, and usually cleared spontaneously by the host's immune system. For example, Lai et al. [2] reported 49% clearance of HPV infection after 3 years. The high clearance rate that contributes to the global prevalence of HPV infection has been estimated at approximately 11.7% in a meta-analysis of 1 million women between the ages of 18 and 69 years. The highest prevalence (over 20%) was found in sub-Saharan Africa and Eastern Europe in comparison with North America and Western Europe, where the prevalence of infection is lower [3].

HPVs belong to the highly diverse papillomaviridae family and over 120 genotypes have been identified so far. HPVs have a high degree of host cell tropism and a productive infection only occurs in keratinocytes of the skin or mucosal surfaces. Mucosal HPV types are subdivided into low-risk and high-risk types according to their propensity to cause cancer [4]. Low-risk types are commonly associated with benign lesions, such as genital warts, whereas high-risk mucosal types, such as HPV-16 and HPV-18, are associated with lesions that have a certain tendency to become malignant. HPV-16 causes approximately 50% of cervical cancer cases, whereas HPV-18 is considered to be the

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second most important HPV type, responsible for approximately 15% to 20% of cervical cancers [5]. High-risk HPVs have been implicated in a number of human squamous cell carcinomas besides cervical cancer including vulvar, anal, and a subset of oropharyngeal carcinomas as well as certain types of skin cancer [6].

HPVs contain a double-stranded circular DNA genome of approximately 8,000 base pairs. The HPV open reading frames encode early (E) and late (L) genes that are required for viral genome replication and virion assembly [7]. The L1 and L2 genes encode viral capsid proteins and are the main targets of vaccination strategies against HPV infection [8]. After infection, the virus establishes itself in replicating host keratinocytes in an episomal form. Upon differentiation, L genes, such as L1 and L2, are expressed and virions are sloughed off with the superficial epithelial layers [4]. High-risk HPV E6 and E7 function as the major oncoproteins of high-risk HPVs and alter the function of critical host cellular proteins [7]. High-risk HPV E6 is known to promote the degradation of the p53 tumor suppressor protein, whereas high-risk HPV E7 was shown to bind and degrade the retinoblastoma tumor-suppressor protein (pRB) (Fig. 1) [7,9]. This subversion of 2 central host tumor-suppressor pathways is crucial to promote viral replication in differentiating host keratinocytes as the virus relies on the host cell DNA replication machinery for viral genome replication [10]. Integration of the HPV genome into a host cell chromosome is commonly preceding malignant progression and only the HPV E6 and E7 open reading frames are maintained at the integrated state. Viral integration terminates the viral life cycle and production of infectious viral particles. HPVs do not enter viral latency and there is no reactivation from the integrated state of HPV infection, although the concomitant presence of integrated and episomal viral DNA has been reported [11]. In contrast to high-risk HPV types, low-risk HPVs, such as HPV-6 and HPV-11, which do not cause cancer, also do not degrade or have a significantly reduced affinity to p53 and pRB, respectively.

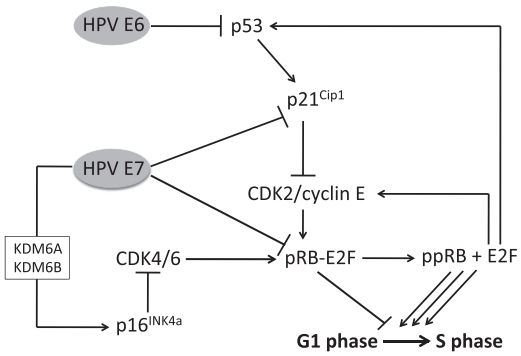

Fig. 1. High-risk HPV oncoproteins E6 and E7 interact with host suppressor proteins and disrupt regulatory nodes that control the cell division cycle. The high-risk HPV E6 oncoprotein stimulates the degradation of p53 thereby thwarting p53-mediated checkpoint control. The high-risk HPV E7 oncoprotein binds and degrades the pRB tumor suppressor protein and inactivates $p21^{Cip1}$, resulting in a relaxation of cell cycle checkpoint control and unscheduled G1 to S phase transition of the cell division cycle. High-risk HPV E7 also reprograms the histone demethylases KDM6A and KDM6B, which stimulate the expression of $p16^{INK4a}$, an inhibitor of CDK4/6 and a widely used tissue-based marker for HPV-transformed cells.

Screening for HPV infection has long relied exclusively on the Papanicolaou (Pap) test, the oldest and most widely used screening tool for cervical neoplasias and their precursor lesions. It involves cytological examination of organ-derived cells (smear), which makes it subject to interobserver variability, sample quality variations and false-negative results. Recently, immunohistochemistry (IHC) for $p16^{INK4a}$ has been introduced as a supportive tool to increase the diagnostic accuracy as this cyclin-dependent kinase inhibitor is overexpressed in virtually all HPV-transformed cells [12].

An alternative and supplement to the Pap test for screening purposes is the detection of HPV DNA. In comparison with the Pap test, HPV DNA testing is less dependent on personnel training and more objective. HPV DNA can be detected by Southern hybridization, *in situ* hybridization, (ISH) and a variety of polymerase chain reaction (PCR)-based methods. Southern hybridization has limited use for HPV testing as it requires large amounts of DNA and is labor intensive. HPV DNA ISH is based on the use of labeled probes that specifically hybridize to HPV DNA. Most recent studies employ PCR-based methods owing to its high specificity and sensitivity. Both type-specific and general PCR can be used for the detection of an HPV infection. However, using PCR for detection of HPV infection also has its shortcomings and may, at least to a certain extent, explain discrepancies between various reports. Issues associated with the use of PCR for HPV detection are sample contamination and the choice of primers for the PCR reaction [13].

An additional approach for the detection of an HPV infection is serology. An infection with HPV is followed by humoral immune response and can be detected by the presence of antibodies to capsid proteins [14]. For the last 2 decades, enzyme-linked immunosorbent assays (ELISAs) have been extensively used in HPV serology. This method allows the detection of conformational and type-specific epitopes, which are present on assembled HPV capsids [15]. The fact that viral integration leads to the loss of large part of the viral genome has obvious implications for HPV detection. The detection of high-risk HPV E6 or E7 mRNA may help to identify significant disease, whereas L1 antibody will, at the integrated stage when virions are no longer produced, only reflect past infections.

Decades of research on cancers caused by viruses have shown that it is critical to base such a connection on an array of methodologies and approaches [16]. Fig. 2 summarizes the aspects that need to be taken into consideration, which range from the physical detection of viral genes and gene products in a lesion to model systems, serology, and epidemiology.

Besides anogenital, cutaneous, and oral squamous cell carcinomas, high-risk HPVs have also been implicated in