



Therapy of HPV-related Cancers with CoVal™ Fusions

Introduction

Human papillomavirus (HPV) infection is the most common sexually transmitted disease and is second only to human immunodeficiency virus (HIV) infection in causing morbidity and mortality. One of the principle modes of HPV transmission is by sexual contact. HPV transmission is not precluded by condoms and it has been estimated that over 70% of the sexually active population in the United States has been infected with HPV (1). HPV specifically infects the squamous epithelium and is associated with a number of benign or malignant diseases of the skin and mucosal surfaces. There are over 100 genotypes of HPV, classified as high or low risk depending on their association with benign or malignant disease. Low-risk HPV types are associated with a variety of warts (papillomas), such as common skin warts and anogenital warts. For example, genital warts are caused primarily by HPV types 6 and 11. High-risk HPV types are associated with anogenital dysplasias (precancerous lesions) and anogenital cancer, including cervical, vulvar, vaginal, penile and anal cancer. For example, HPV type 16 is strongly associated with anogenital cancers, especially cervical, and is frequently implicated in a variety of anogenital dysplasias.

Cervical Cancer

Cervical cancer is the second most common cancer in women worldwide (2). Incidence rates vary from about 10 per 100,000 per year in many industrialized nations to more than 40 per 100,000 in some developing countries. Four of five new cases are currently diagnosed in the developing parts of the world (3). The incidence of cervical cancer is significantly lower in the industrialized world than in developing countries primarily because of widespread screening for cervical HPV infection using the Papanicolaou (Pap) smear test. Nonetheless, despite this public-health vigilance, in 2002 it was estimated that 13,000 cases of invasive cervical cancer would be diagnosed in the United States and that 4,100 women would die from this disease (4). Equally significant is that stage-specific survival of cervical cancer patients has not improved since the 1960s. As a result, the 5 year survival rate for the average cervical cancer patient in the U.S. is only 67% (5). High-risk types of HPV are present in over 99% of cervical cancers, confirming HPV as a necessary cause of invasive cervical cancer worldwide (6). Notably, HPV16 DNA has been detected in 50% of cervical cancers (7). Cellular transformation by high-risk HPV types is primarily due to disruption of the cell cycle controllers, p53 and Rb by the viral E6 and E7 proteins, respectively. Maintenance of the transformed state requires continued expression of these viral oncoproteins, which can be further enhanced by

chromosomal integration of the HPV genome and concomitant loss of viral E1/E2 mediated control of E6/E7 gene expression (8). In addition to infection with high risk HPV, additional risk factors for the development of cervical cancer include smoking, immunosuppression, hormonal factors and genetic predisposition (9,10).

Anal Cancer

Anal squamous cell carcinoma (ASCC) is increasing in frequency in the general population in the US, Europe and South America. The incidence of ASCC has been increasing over the past 30 years in the US with an estimated 3,900 new cases in 2002 (4). For example, between 1973 and 2000 the incidence of anal cancer in men and women in the US increased 160% and 78% respectively (11, 12). In the US and Europe, this increase has been observed most notably among women, unmarried men and persons living in or near large cities (13-15). This trend has been most evident in urban populations with large concentrations of homosexual men, such as San Francisco, where the incidence of anal cancer in men aged 40-64 years increased more than 5-fold between 1978 and 1999 (16). Thirty years ago, anal cancer was believed to be caused by chronic, local inflammation of the perianal area. However, as for cervical cancer, HPV is now recognized as a principal cause of anal cancer (17). Given the paucity of research on the role of HPV in the development of anal cancer, much of the information is extrapolated from the abundant literature on HPV infection in cervical dysplasia and cancer. This extrapolation is reasonable given the strong similarities in histology and histopathology of the tissues and malignancies involved.

In a cohort of patients from Denmark and Sweden, high-risk HPV was detected in nearly 90% of anal cancer biopsies, similar to findings globally in cervical cancer (17). Furthermore, increased risk for anal cancer has been linked to the presence of other HPV-associated diseases, such as cervical cancer, vaginal cancer and anogenital warts (18-21). As is the case for cervical disease, HPV16 is the subtype most frequently associated with high-grade anal dysplasia and anal cancer, and in two independent studies was found in 73% of patients with anal cancer (17, 22, 23). Epidemiologic surveys have implicated genital viral infections and sexual practices in the pathogenesis of anal cancer. As expected for a disease associated with STD transmission, the sexual activities of women and heterosexual men were related to risk for developing anal cancer (17). The relative risk of anal cancer in women was highest for those with many sexual partners, a history of receptive anal intercourse, a history of anal warts, genital warts and cervical dysplasia or other STDs. Among heterosexual men, elevated risk for anal cancer was associated with many sexual partners, a history of anal warts or other STDs.

In both men and women, HIV positivity is a significant risk factor for anal cancer, regardless of sexual practices (22, 24-27). The incidence of ASCC is significantly higher among homosexual men and more so among those HIV-positive than in

the general population. For example, in Denmark, where same-sex partnerships are legally sanctioned, one study found that the relative risk (RR) of invasive anal cancer in homosexual men and women who had registered their relationship was 31.2 compared with the general population (28). Similarly, by matching cancer and AIDS registries another study found an almost 40-fold increase in the RR of anal cancer in men and a nearly 7-fold risk in women, which was greater than the 5.4-fold increase in risk for cervical cancer (29). Current estimates for the incidence of ASCC in homosexual men are up to 35 per 100,000 for those that are HIV negative, which approaches the incidence of cervical cancer in women prior to the institution of regular Pap smear screening (15, 30, 31). In HIV-positive homosexual men, the incidence of ASCC is approximately double that seen in HIV-negative homosexual men (32). Therefore, HIV infection increases the risk of HPV infection and progression of anal dysplasia to anal cancer.

Presently, it is unclear whether HIV infection has a direct effect on the development of anal cancer. In some studies, HIV-induced immunosuppression, measured as CD4 lymphocyte count, appears to play a role (22, 33), while another study found the standard incidence ratios of cervical and anal cancer were not significantly associated with CD4 counts (34). Intriguingly, one study found that highly active antiretroviral therapy (HAART) was associated with substantial declines in opportunistic infections and the AIDS-defining malignancies Kaposi's sarcoma and non-Hodgkin's lymphoma but not rate of HPV infection or progression to HSIL (35). This suggests that HPV may evade the form of immune surveillance reconstituted by this therapy. Despite the ambiguities in the HIV data, an important role for immunosurveillance is emphasized by the dramatic increase in risk (100-fold) for anal cancer observed in immunosuppressed renal transplant recipients (36, 37).

Given the propensity of high risk HPV types to cause malignant transformation of epithelial cells and the prevalence of HPV in the general population, it is not surprising that HPV is suspected in the etiology of other cancers. Indeed, a number of clinical studies have demonstrated the presence of HPV DNA in tumor biopsies from a number of anatomical locations outside the anogenital region.

Head and Neck Cancer

Head and neck squamous cell carcinoma (HNSCC), which includes cancers of the oral cavity, pharynx and larynx, occurs with an annual incidence of approximately 45,000 cases in the United States (38). HNSCC is a disease typically associated with environmental risk factors such as tobacco use and alcohol consumption (39, 40). However, a significant proportion occurs in individuals without these risk factors. Recent molecular and epidemiologic evidence indicates that HPV infection of the upper airway likely promotes head and neck tumorigenesis (41, 42). As in anogenital disease, infection with high risk HPV, most frequently type 16, is implicated in a subset of HNSCC. As in other HPV-associated cancers, the viral oncoproteins E6 and E7 promote tumorigenesis by disrupting the p53 and Rb cell-cycle pathways previously

identified as being important in the progression to HNSCC (43, 44). Studies in different ethnic populations have demonstrated that approximately 25% of HNSCC cases are HPV-associated (45-47). In a literature survey, an average of 34.5% of head and neck cancers were HPV positive by polymerase-chain reaction (PCR) assay (48). As for anogenital infections, HPV16 positivity predominates in HNSCC, apparently even more so. For example, in two studies, of the HPV positive head and neck tumors, 84% to 90% contained HPV16 (47, 49). In most studies, HPV presence is strongly associated with oropharyngeal tumors, and in one study 94% of the HPV positive tumors were tonsillar in origin (49).

Strong evidence for a causal involvement of HPV in a subset of HNSCC comes from the detection of E6/E7 expression and high viral DNA load in tonsillar carcinomas (50-52). Consistent with these observations, E6/E7 mRNA expression was associated with reduced levels of Rb and overexpression of the Rb-regulated protein p16^{INK4a} in a study of HPV16 positive HNSCC (53). Epidemiologic support for the involvement of HPV in tonsillar cancer comes from the observation that individuals with a history of HPV-associated anogenital malignancy are at increased risk for a second primary cancer arising from the tonsils (54). Within the oropharynx, HPV-positive cancers were less likely to occur in smokers and alcohol drinkers, were less likely to have tobacco-associated p53 mutations, possessed a distinct basaloid histopathology previously observed in HPV-positive penile, vulvar and anal cancers, and were associated with a more favorable prognosis. These data strongly suggest that HPV-associated oropharyngeal cancers are a distinct clinical entity (45). The risk of tonsillar cancer is also increased among HIV-infected men, as are other HPV-related penile, anal and conjunctival SCCs (29). Tonsillar carcinomas have also recently been reported in a posttransplant setting (55, 56), analogous to the persistence of HPV infection and elevated risk for anogenital cancers in immunosuppressed HIV+ individuals (29, 57, 58).

The role that HPV plays in head and neck cancer outside of the oropharynx is less clear. In PCR-based studies conducted in Japan, Italy, France and India, HPV positivity in HNSCC samples from the oral cavity, pharynx and larynx ranged from 16 to 73%, and as with oropharyngeal HNSCC, HPV16 was the most frequently detected genotype (59-62). Additional support for an etiologic role for HPV in the pathogenesis of HNSCC comes from studies demonstrating the presence of HPV in precursor lesions with malignant potential. In one study, using a highly sensitive nested PCR technique, 25/29 cases of hyperplasia (86%), 5/5 cases of dysplasia (100%) and 16/18 invasive oral SCCs (89%) were positive for high risk HPV16, -18 or -33 (63).

Other HPV associated Cancers

In the anogenital cancers discussed above, the weight of evidence favoring a causal role for HPV infection in tumorigenesis is compelling, and in the case of cervical cancer, is incontrovertible. Using similar etiologic principles, high-risk

HPV infection, particularly type 16, is likely responsible for a subset of head and neck cancers. In the search for additional cancers which may be related to HPV infection, researchers have detected the presence of HPV DNA sequences in biopsy samples from several types of cancer using in situ hybridization and/or PCR techniques. These include, for example, lung and non-melanoma skin cancer (64-67). It should be cautioned that many of these studies represent preliminary molecular evidence for the presence of specific HPV DNA sequences in isolated tumor tissue. Given that HPV is ubiquitous in the general population, additional molecular, virologic and epidemiologic evidence is required to infer a direct role for HPV gene expression in the development of these other cancers.

Interestingly, in certain skin cancers, additional molecular evidence implicates HPV in tumorigenesis. Non-melanoma skin cancer (NMSC) is the most frequently occurring malignancy worldwide in the Caucasian population. Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are types of NMSC that occur primarily on sun-exposed skin, implicating ultraviolet (UV) radiation in their development. The ratio of BCC to SCC is 5:1 in immunocompetent populations, whereas in the immunosuppressed, the risk for SCC development is 250 times greater than in the general population (68). Such immunosuppressed populations include renal transplant recipients and patients with the rare inherited disease epidermodysplasia verruciformis (EV). A subset of cutaneous HPV types are associated with EV, with HPV types 5 and 8 predominating (69). In the case of EV, evidence that strengthens the role of HPV gene expression in tumorigenesis comes from the demonstration of the oncogenic potential of the early genes of HPV5 and 8 in vitro and of HPV 8 in vivo (70, 71). Therefore, in this type of cutaneous cancer, HPV gene products such as E6 and E7 may transform epithelial cells in combination with the known carcinogenic effects of UV light.

Therapeutic Vaccines to Treat HPV-associated Cancer

A role for the immune system in host defence against HPV comes from a variety of sources, including the study of HPV-infected humans and in animals infected with other types of papillomavirus. Studies on the natural history of HPV infection clearly indicate that the immune system plays an important role in controlling this virus. For example, compared to the large number of individuals exposed through sexual activity to HPV, only a small percentage develop cancer. For most, infection is transient and elicits an immune response which, by currently available tests, apparently clears their infection (72). Similarly, although a general feature of many types of HPV-induced lesions is their tendency to recur or persist, the spontaneous regression of lesions associated with low-risk HPV is not uncommon and is thought to reflect host immune responses. Conversely, the incidence of HPV infection is greatly increased in immunocompromised renal transplant recipients and HIV+ individuals (73, 74). Supporting these observations are studies implicating cellular immunity in the resolution of papillomavirus induced lesions. For example, in the canine oral papillomavirus

(COPV) and cottontail rabbit papillomavirus (CRPV) models, and in anogenital warts in humans, regressing wart lesions are infiltrated with T cells and macrophages (75-77). Despite this evidence, correlating the natural human immune response to resolution of HPV-mediated disease has proved challenging, perhaps because important local immune responses in the anogenital mucosa are not reflected systemically. Hence, the presence of a cytotoxic T lymphocyte (CTL) response to HPV early antigens has been correlated to clearance or absence of anogenital HPV lesions in one study (78), while in another, the relationship between CTL activity and disease state was unclear (79).

In contrast to natural history studies in humans, the well-characterized animal models of HPV infection have permitted researchers to characterize the types of immune responses associated with disease regression. In particular, in murine models of HPV-associated cancer, therapeutic vaccine studies have clearly demonstrated the importance of T cell responses and especially those of CD8+ CTL in tumor rejection (reviewed in 80). Therefore, a variety of preclinical and clinical evidence points towards the importance of cellular immune responses in eradicating HPV-associated lesions, such as warts, dysplasia and cancer. In light of this, it is noteworthy that Stressgen researchers and collaborators have demonstrated in preclinical models that CoVal™ fusions elicit strong cellular immunity, characterized by induction of CTL, production of Type 1 cytokines and activation of dendritic cells (see “CoVal™ Fusions and HspE7” section for a detailed discussion).

Stressgen has taken the CoVal™ fusion concept from the laboratory into phase II and phase III clinical trial testing and has demonstrated significant activity of its lead CoVal™ fusion, HspE7, in the therapy of HPV-associated diseases (clinical trial results are discussed in the “CoVal™ Fusions and HspE7” section). Stressgen is currently seeking partners to participate in the development of HspE7 for HPV-associated cancers.

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