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Viruses and neoplastic growth

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The relationship between viruses and neoplasia has intrigued researchers for decades. Establishing the pathogenetic process by which viruses act as either primary factors or cofactors is important to both prevention and treatment of neoplasia. Although numerous studies have clearly established viruses as etiologic agents in animal tumors, the identification of tumor viruses in humans has been more complex. Traditionally, the application of Koch's postulates is needed to demonstrate that a specific microbiologic agent is responsible for an infectious disease. It has not been possible to apply Koch's postulates to the viral etiology of human cancers because viral agents appear to be host specific and cannot be maintained under tissue culture conditions [1]. Consequently, it has become necessary to develop different criteria for establishing the viral etiology of human neoplasms from those used traditionally to establish the microbial etiology of infectious diseases. These criteria include the following [1]:

- The regular presence and persistence of the respective viral DNA in tumor biopsies and cell lines derived from the same tumor type
- The demonstration of growth-promoting activity of specific viral genes or of virus-modified host cell genes in tissue culture systems or in suitable animal systems
- The demonstration that the malignant phenotype depends on the continuous expression of viral oncogenes or on the modification of host cell genes containing viral sequences
- Epidemiologic evidence that the respective virus infection represents a major risk factor for cancer development

Viruses are classified into DNA and RNA types. Viruses that cause neoplasia are called oncogenic (tumor-producing) viruses. Studies have shown that DNA-type and RNA-type viruses have oncogenic potential.

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Evidence supporting the viral etiology of certain human cancers is increasing. Approximately 15% of human cancers can be linked to viral etiology (Table 1) [1]. The DNA viruses that have been implicated in the etiology of human neoplasms include human papillomaviruses (HPVs), Epstein-Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), Kaposi's sarcoma–associated herpesvirus (KSHV), and herpes simplex virus 2. Among the RNA tumor viruses or retroviruses, only human T-lymphotrophic 1 (HTLV-1) has been clearly associated with human tumors [2].

Results of studies examining the primary and secondary effects of viruses in neoplastic pathogenesis include epidemiologic evidence and experimental results that suggest modes of interaction [1]. The epidemiology pattern of some human tumors is consistent with an infectious etiology [1]. For example, numerous epidemiologic studies have linked cervical cancer to either female sexual promiscuity or being a monogamous female partner of a promiscuous husband. High-quality molecular tests for HPV DNA have strengthened this association between HPV and cervical neoplasia [3]. Studies of HIV-associated Kaposi's sarcoma suggested epidemiologic patterns that were consistent with a sexually transmitted agent before a viral agent (KSHV) was identified [4,5]. Other epidemiologic studies have suggested an infectious etiology for Hodgkin's disease, some non-Hodgkin's lymphomas, and childhood leukemias [1].

The pathogenesis of neoplasia is a multistep process. The potential of certain viruses to become involved in the etiology of tumors is due to their ability to incorporate their viral genome into that of a host cell [2]. Viral participation in that process includes the direct involvement of viral genes, the activation of oncogenes (altered versions of normal genes that regulate

Table 1
Viruses linked to human cancers

Viruses	Cancers
DNA viruses	
Human papillomaviruses	Anogenital cancers
	Cancers of the skin
	Oral verrucous carcinoma
	Oral squamous cell carcinoma
Epstein-Barr virus	Burkitt's lymphoma
	Nasopharyngeal carcinoma
	Hodgkin's lymphoma
	Thymic lymphoepithelial carcinoma
	Immunosuppression-related lymphomas
	Squamous cell carcinoma
Human herpesvirus 8	Kaposi's sarcoma
Hepatitis B virus	Hepatocellular carcinoma
Hepatitis C virus	B-cell non-Hodgkin's lymphoma
RNA viruses (retroviruses)	
Human T-lymphotrophic virus 1	Endemic T-cell leukemia/lymphoma

normal cell growth and differentiation), and the inactivation of tumor suppressor genes [6]. In vitro studies have demonstrated that DNA and RNA viruses are able to establish persistent infections and thereby transform or immortalize cells. From in vitro studies, characteristics of transformed or immortalized cells have been described. These characteristics include an increased cell growth rate with loss of cell growth inhibition, continued cell growth without senescence, and changes in cellular morphology and metabolism. Demonstration of direct viral oncogenesis in human tumors is not as clear as in vitro studies. The mechanism of virally associated tumor formation is variable and complex. Oncogenic viruses use different processes to increase cell growth rate. These include providing factors that stimulate cell growth, inactivating cell regulatory proteins, and preventing cell death by apoptosis. The mechanism used by some viruses to stimulate cell growth may relate to the position at which the viral genome is integrated into the cellular genome or to the state of differentiation of the infected cells. There is evidence for direct oncogenesis, evidence for tumor formation by stimulation of cellular genes that have the potential to change into a gene that can cause the cell to become a neoplastic cell (proto-oncogene) and, in the association of HBV and HCV with tumor formation, evidence for the stimulation of liver cell growth resulting from persistent infection, tissue destruction, and repair. KSHV, also known as human herpesvirus 8, promotes the development of Kaposi's sarcoma using growth-promoting cytokines encoded by the virus. Further complicating the etiologic association between viruses and neoplasia, immortalized cells are more likely than normal cells to accumulate other mutations or chromosomal rearrangements and may be more susceptible to cofactors and tumor promoters [2].

The aim of this article is to review information that describes relationships between viruses and neoplasia. The viruses included here are HPVs, EBV, KSHV, HBV, HCV, and retroviruses.

DNA oncogenic viruses

Human papillomaviruses

HPVs are found in the epithelium of skin and mucosa, and more than 100 HPV types have been identified. Establishing the relationship between HPVs and neoplasia is complex because the virus may be present in the epithelium of individuals with no HPV-associated lesion. The prevalence rates in individuals with no lesions range from 0% to 60% [7].

Further complicating the studies of the relationship between HPVs and neoplasia is the need for different probes to specifically identify the different HPV types present in tissue. Despite these difficulties, epidemiologic and virologic evidence supports an etiologic relationship between HPVs and neoplasia.

HPVs have been clearly established as etiologic agents in neoplasia of the vaginal cervix [3]. HPV types have been identified in benign and malignant oral epithelial lesions. Among the benign lesions are oral papillomas, verruca vulgaris, condyloma acuminatum, and focal epithelial hyperplasia [8]. Among the premalignant and malignant lesions are viral-induced leukoplakia, proliferative verrucous leukoplakia, and erythroplakia [8]. An HPV-associated dysplastic epithelial lesion called koilocytic dysplasia has been described by Fornatora et al [9]. HPV has been identified in oral verrucous carcinoma and squamous cell carcinoma [8].

As described earlier in this article, the potential of certain viruses to become involved in the etiology of tumors is due to their ability to incorporate their viral genome into that of a host cell [2]. HPVs access and infect the cells of the epithelial basal cell layer through breaks in the skin or mucosa. The virus replicates during keratinocyte differentiation in the spinous and granular cell layers [10]. HPVs are able to modify cellular genes by either functionally inactivating them or targeting them for degradation [1]. A portion of the HPV genome encodes proteins that are capable of inducing cell proliferation and transformation [3]. Proteins are expressed in all HPV-associated lesions from benign lesions with no malignant potential to high-grade invasive carcinomas [3]. In benign HPV-infected lesions, however, the viral DNA exists as extrachromasomal plasmids, whereas in most cancers, the HPV DNA is integrated into host chromosomes [3]. This integration into host cell proteins is responsible for the disruption of the transcription of regulatory proteins, resulting in neoplastic deregulation.

The malignant potential of HPV varies from low (HPV types 6 and 11) to high (HPV types 16,18, 31, and 33) [3]. Low-risk viruses are defined as those that are almost never found in invasive cancers and high-risk viruses are those that are most often found in invasive cancers [3]. In the laboratory, DNA from high-risk HPV types is capable of epithelial cell line transformations, unlike DNA from low-risk types. These transformations, however, require passage through many cell generations before becoming apparent [3]. The integration site of HPV in the host cell genome has been found to be in the general region of known oncogenes, including c-*myc*, a gene expressed in all cells that has the potential to change into an active oncogene, and suggests that HPV high-risk types may activate transcription of cellular oncogenes. Other studies have demonstrated the inhibition of tumor suppressor genes by HPV [3].

The ability of HPV oncogenes to be expressed depends on the state of differentiation of infected keratinocytes [1]. The interference with cellular control due to infection of basal cells is important both in cell immortalization and in malignant transformation and results in hyperplasia of the basal and prickle cell layers and dysplastic cellular changes [2] (Fig. 1). A high percentage of squamous cell carcinomas of the skin contain HPV. There is evidence that some HPV types present in skin can prevent apoptosis and permit the survival of cells damaged by ultraviolet radiation that would

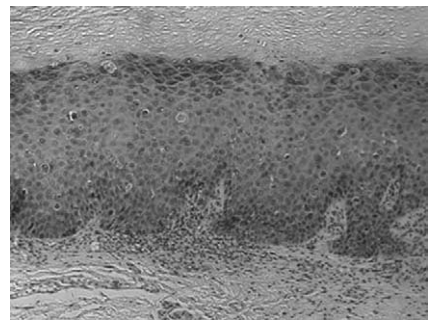

Fig. 1. HPV-induced epithelial hyperplasia and dysplasia (original magnification × 10; hematoxylin and eosin stain).

otherwise be prone to apoptosis, thereby allowing cells with genetic mutations to survive and flourish.

The characteristic cells of HPV lesions are the koilocytes: enlarged squamous epithelial cells with clear halos around shrunken nuclei (Fig. 2). A portion of the HPV genome encodes a protein that binds to and disrupts the cytoplasmic keratin network producing the koilocyte [3]. HPV, however, may be present in epithelium, even in the absence of koilocytic epithelial cells [3].

There is epidemiologic and molecular evidence supporting the role of HPV types 16 and 18 in the etiology of cervical cancer. The role of HPV infection in the pathogenesis of head and neck cancer is not as clear [11]. Fornatora et al [9] described a histologic form of oral epithelial dysplasia (koilocytic dysplasia) clearly associated with HPV and characterized by the presence of koilocytes and epithelial dysplasia. The potential for malignant transformation of these lesions is unknown [9].

Verrucous carcinoma is characterized by exophytic, noninvasive tumor growth. Tobacco use has been demonstrated to be an important etiologic agent [12]. Results of HPV identification include low-risk and high-risk

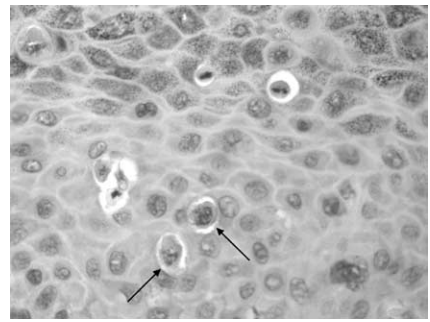

Fig. 2. Koilocytes (original magnification × 40; hematoxylin and eosin stain).

types, but these findings are inconsistent [8]. In oral squamous cell carcinomas, the finding of HPV has also been inconsistent, with rates ranging from 0% to 100%. As with verrucous carcinoma, low-risk and high-risk types have been identified [7,8]. The relationship between these findings and the pathogenesis of these neoplasms remain unclear. The vast differences reported may, in part, be a result of the different methods and different types of tissue used in these studies [7]. Alcohol and tobacco use are important factors in the development of oral squamous cell carcinoma; however, a proportion (15%–20%) of patients with oral squamous cell carcinoma have no history of exposure to these factors [11]. In a large series of head and neck cancer patients, Gillison and Shah [11] found HPV DNA in 24.5% of head and neck cancer specimens; HPV 16 was identified in 90% of these. Most of the HPV-positive tumors were located in the pharyngeal and lingual tonsillar area, and the patients with these findings were less likely to have had chronic exposure to tobacco and alcohol. Molecular and histopathologic characteristics of these tumors suggested that HPV-associated tonsillar carcinomas may be a distinct clinical subtype [11]. It has also been suggested that there may be two parallel or overlapping pathogenic pathways of oral squamous cell carcinoma: one etiologic pathway related to exposure to substances such as tobacco and alcohol and another related to an infectious agent [13]. In an overlapping pathway, carcinogens such as tobacco may act as cofactors, resulting in full oncogenic expression of HPV [10]. The finding of HPV in certain smokeless tobacco–associated white lesions and verrucous carcinoma may be examples of the interaction of tobacco carcinogens and HPV.

Although the relationship between HPV and oral lesions remains unclear, evidence from studies of carcinoma of the uterine cervix and results of oral studies that suggest a relationship between these viruses and oral neoplasia justify continued study of the oncogenic role of HPVs in oral neoplasia.

Epstein-Barr virus

EBV is a member of the human herpes virus group and is associated with lymphoid and epithelial neoplasms including endemic Burkitt's lymphoma, immunosuppression-associated lymphomas, Hodgkin's disease, nasopharyngeal carcinoma, thymic lymphoepithelial carcinoma, and squamous cell carcinoma [6]. Establishing either a direct, indirect, or cofactor etiologic relationship between EBV is even more difficult than HPV because of the ubiquitous presence of EBV in the general population. Approximately 90% of adults have demonstrable EBV antibodies and, therefore, the presence of EBV in some tumors may be coincidental [14]. The association of EBV with epithelial and lymphoid neoplasms is consistent with the results of studies of EBV infection pathogenesis that show a tropism toward lymphocytes and epithelial cells [6]. Different EBV genes may be responsible for epithelial trophism than for lymphotropism [6].

EBV, like other herpesviruses, kills the cells in which it replicates and is able to establish a persistent latent infection. With EBV, the initial infection is in epithelial cells and the latent infection is in B lymphocytes. EBV is transmitted in saliva and initially infects oropharyngeal epithelial cells. In epithelial cells, the viral genome can be identified in the basal and parabasal layer of epithelium. The virus replicates and is released from epithelial cells. Virus released from epithelial cells infects B lymphocytes present in the lymphoid tissue surrounding the posterior oral cavity and oropharynx [14]. The latent infection of B lymphocytes remains for the life of the host [6]. In vitro evidence demonstrates that EBV infection results in the induction of viral oncogenes that affect host cell proteins [1].

Findings associating EBV with lymphoid neoplasia are inconsistent. EBV is not uniformly present in non-Hodgkin's lymphoma; however, when EBV proteins are expressed, they are expression of latent EBV genes with oncogenic potential [14]. In a study of oral non-Hodgkin's lymphomas, Leong et al [14] found that EBV was common in immunosuppression-related oral lymphomas and rare in other non-Hodgkin's lymphomas, suggesting that the virus is likely more important in the pathogenesis of some lymphomas than in others [14]. EBV has been found in undifferentiated nasopharyngeal carcinoma and is weakly associated with gastric carcinoma.

Kaposi's sarcoma–associated herpesvirus

KSHV is also called human herpesvirus type 8. The evidence linking this virus to neoplasia, particularly Kaposi's sarcoma, is substantial. KSHV has been consistently detectable in several different epidemiologic forms of Kaposi's sarcoma: the classic sporadic form that primarily affects elderly males, the more aggressive endemic type seen in Central Africa that affects children and young men, the post-transplantation type of Kaposi's sarcoma that is a rare complication of immunosuppressive therapy, and HIV-associated Kaposi's sarcoma [15]. In HIV-associated Kaposi's sarcoma, oral mucosal lesions are common (Fig. 3). KSHV is not ubiquitous in humans, and the results of epidemiologic studies strongly suggested a transmissible agent even before the virus was consistently identified in these lesions [4,5]. KSHV infection rates in the United States are less than 3% and may approach 50% in some Central African countries [15].

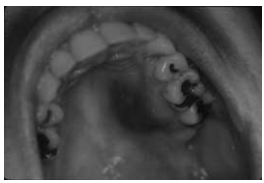

Fig. 3. Kaposi's sarcoma on the palate of an HIV-seropositive man.

The role of KSHV in the pathogenesis of Kaposi's sarcoma and other neoplasms is far from clear. The oncogenic mechanisms that involve KSHV infection appear to involve multiple steps and be dependent on the interaction of KSHV with other viruses such as HIV and EBV and with other factors [16]. For example, one of the HIV proteins (tat) has been shown to act as both an angiogenic factor and a stimulator of KSHV replication. Genetic interactions are suggested by the prevalence of Kaposi's sarcoma in certain ethnic groups. Men are far more likely to develop Kaposi's sarcoma than women, suggesting an influence by male hormones.

Human keratinocytes support KSHV infection, and mucosal epithelium may be the primary site of infection. KSHV is found in the normal skin of patients infected with HIV who have Kaposi's sarcoma. Like other herpesviruses, primary KSHV infections are followed by a lifelong latent infection. The latent infection is maintained in infected B lymphocytes [17]. The ability of KSHV to cause neoplastic transformation is due to its capability to transform and immortalize the cells it infects, upset the balance between growth activators and suppressors, and inhibit apoptosis [15].

Hepatitis B virus and hepatitis C virus

Hepatocellular carcinoma is one of the most frequent visceral neoplasms worldwide. It almost always develops in individuals with chronic hepatitis or cirrhosis. HBV and HCV are considered main causative agents in the development of hepatocellular carcinoma [18]. The process that culminates in the development of hepatocellular carcinoma may take more than 30 years after infection with HBV or HCV [18]. The specific mechanism by which HBV and HCV are involved in the development of hepatocellular carcinoma is poorly understood [1,18]. It is not entirely clear whether the effect of these viruses is direct or indirect, and differences exist between the effects of HBV and HCV on the hepatocyte genome. Rather than a direct oncogenic effect, they may, for example, act as indirect carcinogens due to the induction of the reactive oxygen radicals produced in chronic inflammation [1]. Furthermore, specific alterations in the hepatocyte genome, including chromosomal losses and gains, are caused by HBV and HVC and appear to increase as hepatocytes become dysplastic.

Relationships between HCV and other cancers have been suggested. Reports have linked HCV to the etiology of non-Hodgkin's lymphoma, but these findings are inconsistent [19]. Studies have shown a higher prevalence of HCV infection in patients with B-cell non-Hodgkin's lymphoma. HCV may increase the risk of non-Hodgkin's lymphoma by activating B lymphocytes, resulting in secretion of IgM and subsequent B-cell stimulation [19].

Herpes simplex virus and cytomegalovirus

Two other herpesviruses, herpes simplex virus and cytomegalovirus, although not directly associated with neoplasia, have been shown to

enhance the persistence and replication of other viruses. These herpesviruses could potentially be indirectly involved in neoplasia [1].

RNA oncogenic viruses

Human T-lymphotrophic virus type 1

RNA viruses linked to oncogenesis are retroviruses. Retroviruses are thought to be involved in the pathogenesis of a wide range of diseases including autoimmune diseases and neoplasia. Several retroviruses have been clearly linked to neoplastic transformation in animals, but only HTLV-1 has been clearly linked to neoplasia in humans. HTLV-1 is associated with an endemic form of T-cell leukemia/lymphoma in parts of Japan and the Caribbean. This neoplasm is also occasionally found elsewhere, including the United States [20]. The pathogenetic mechanism employed by HTLV-1 is not clear; however, studies exploring the role of retroviruses in the pathogenesis of neoplasia are ongoing and these viruses are likely to become increasingly important in our understanding of oncogenes.

Certain characteristic retroviral activities of HTLV-1 are consistent with its function as an oncogene and its role in T-lymphocytic leukemia and lymphoma. Retroviral receptors determine the hosts in which they function and cell and tissue specificity. The retrovirus genome is replicated by a process called reverse transcription. This process occurs almost immediately after the virus attaches and penetrates a cell. The RNA is duplicated and transported to the nucleus where the viral genome becomes integrated into and indistinguishable from the host cell DNA [21]. One of the proteins that results from cellular infection with HTLV-1 has been shown to activate the transcription of host cell genes. Its role in neoplasia is likely related to the activity of HTLV-1 in stimulating the proliferation of T lymphocytes. This T-lymphocytic proliferation results in the release of cytokines that act on macrophages, which in turn induce secretion of other cytokines and result in further T-cell proliferation. The continued proliferation of T cells increases the risk of mutations, ultimately leading to the outgrowth of a monoclonal neoplastic T-cell proliferation [20].

HIV

HIV replicates and becomes incorporated into the cellular genome in much the same manner as described previously for HTLV-1. At present, the role of HIV in neoplasia appears to be primarily related to the tumors that emerge as a result of HIV-induced immunosuppression [1]. The malignancies that are most prevalent in HIV-infected individuals are Kaposi's sarcoma and non-Hodgkin's lymphoma. Early studies of Kaposi's sarcoma in HIV-infected men reported prevalence rates of up 40% [15]. The risk of non-Hodgkin's lymphoma in persons with AIDS is approximately 100 to

300 fold higher compared with the general population [17]. Viruses have been linked to both these tumors: human herpesvirus 8 to Kaposi's sarcoma and EBV to immunosuppresssion-associated non-Hodgkin's lymphoma.

Summary

At present, information concerning the role of viruses in the pathogenesis of human neoplasms is fragmented and incomplete. It is clear that their role is complex, and a complete understanding of the intricacies involved in viral interaction with the human genome may still take many years. New virologic study techniques can be expected to emerge and epidemiologic studies will continue. With each new report, a bit more will be understood, new hypotheses stimulated, and additional studies undertaken. The identification of viral agents as causative agents of neoplasia and the pathogenetic mechanisms by which they act will have a profound effect on our approaches to oral cancer.

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