Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 7619–7621, July 1999

Commentary

Convicting a human tumor virus: Guilt by association?

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More than 15 years have passed since the last printing of John Tooze's classic molecular virology text *DNA Tumor Viruses* (1). Our appreciation of the intricacies of molecular oncogenesis is now even more advanced. Viral infectious agents contribute to a significant fraction of human malignancies worldwide (Table 1). Within the last two and a half years alone, at least three DNA viruses have been linked with various forms of human cancer (2–4). When considering the causal association of a virus with a disease, such as cancer, the burden of proof remains with the biomedical researcher. In a recent issue of the *Proceedings*, Laghi *et al.* (3) provide evidence that JC viral DNA is present in colonic tissue and that an increased number of viral copies is detected in some colon carcinomas. These observations could have implications in understanding of the etiology of colorectal cancers. It is therefore appropriate to consider the rules of evidence required to convict a human cancer virus.

(*i*) A strong epidemiological association of the virus with the disease must be made. Such an association is easiest when the prevalence of the viral agent is low in the human population. Although Epstein–Barr virus (EBV) was the first virus implicated in a human cancer, its high prevalence made it difficult to firmly establish the link between EBV and lymphomas. The identification of EBV occurred in 1964, and this finding was the culmination of years of epidemiological, cell biological, and electron microscopic studies. Burkitt noted in the 1950s that several tumors found in children in Africa resulted from a single malignant lymphoma. He observed that all tribes and races were susceptible, and even though the tumor did not exist in India, it occurred in Indian children who lived in Africa, leading him to suggest that a virus might cause the cancer. Epstein, Achung, and Barr reported that they could continuously culture cells from a Burkitt's lymphoma patient, and, using electron microscopy, they identified a herpes viral particle. EBV DNA is found in ≈90% of Burkitt's lymphoma patients from Africa whereas it is only in ≈15% of these lymphomas from Europe and the United States. However, only when patients with immunosuppression, either by drugs or AIDS, unmasked the polyclonal proliferative syndromes associated with EBV from which a malignant clone containing EBV eventually developed was there a firm conviction of the virus as the etiological agent. Such analyses led ultimately to the association of EBV with some forms of Hodgkin's disease and other types of B cell lymphomas. Acceptance of these findings was facilitated by the long-known effects of EBV, which induces the polyclonal proliferation of immortalized B lymphocytes in culture. EBV also has been epidemiologically linked with nasopharyngeal carcinoma, a disease that predominates in Southern China. Although this evidence implicates EBV as the causative agent of these cancers, it is not the only factor involved. Rather, tumor progression is a multistep process in which additional genetic changes occur and select for the malignant clone.

Another example of a strong epidemiological link between a virus and a human cancer involves the human T cell leukemia virus (HTLV-1). HTLV-1 was the first human retrovirus identified (5). Adult T cell leukemia (ATL) predominates in the islands of Southern Japan, and retroviral particles were isolated from a cell line derived from an ATL patient (6). Although there is a strong geographical association of HTLV-1 with this specific type of cancer, the mechanism of transformation remains unclear. Although the HTLV-1 transcriptional activator tax has been implicated in the transformation of cultured fibroblasts (7), no direct evidence has been provided for the role of this viral gene product in ATL. At this point, the consensus verdict is that HTLV-1 is the etiological agent for ATL. However, as with EBV and lymphomas, it is likely that additional genetic and/or environmental factors participate with HTLV-1 in the pathogenesis of the ATL.

Hepatitis B virus (HBV) infection is a major health problem because there are 300 million carriers worldwide. Up to 50% of the population in parts of southern Africa and southeast Asia have been infected by HBV. There are ≈300,000 cases per year in the U.S., and 0.1–0.5% of the U.S. population are carriers. Chronic active hepatitis is found in ≈25% of the carriers. HBV is a precursor to cirrhosis of the liver and is associated with an increased lifetime risk of developing primary hepatocarcinoma (PHC) (also called hepatocelluar carcinoma), usually 20–30 years later. Many PHC cells contain portions of the HBV genome integrated into their chromosomes, but there is no common viral gene or product persistently expressed. HBV also may synergize with environmental factors such as alcohol-associated cirrhosis in causing PHC. PHC is usually fatal, and there are 250,000 to one million deaths per year worldwide. HBV vaccination initiatives designed to reduce perinatal morbidity and mortality are currently underway. The ultimate proof of the HBV etiology of this cancer will be provided if this vaccination strategy leads to a concomitant reduction in PHC.

Hepatitis C virus was characterized in 1989, and it also is capable of chronic infection. Ten to twenty percent of chronic infections result in cirrhosis, and hepatitis C virus may be involved in primary hepatocarcinoma. For both hepatitis B and C, no direct mechanism of transformation by the virus has been shown. Thus, their strong epidemiologic association with PHC has led to the verdict that HBV and hepatitis C virus act as risk factors for this tumor by perpetuating injury and repair, increasing the probability of genetic aberrations leading to liver cell transformation.

(*ii*) Detection of the viral agent or its products in the diseased tissue should be consistent with a scientifically sound mechanism through which the viral agent facilitates transformation. Recently, a novel herpes virus (HHV-8) was isolated from the lesions of HIV-positive patients with Kaposi's sarcoma (KS) (8). HHV-8 has since been found in non-HIV KS patients. There is a higher prevalence of HHV-8 in some Mediterranean countries compared with Northern Europe and Northern America. This is in agreement with the higher incidence of classical KS in some Southern European coun-

The companion to this Commentary begins on page 7484 in issue 13 of volume 96.

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PNAS is available online at www.pnas.org.

Table 1. Viruses established as etiologic agents or risk factors for human cancers

Viral agent	Clinical disease
Epstein-Barr virus	Burkitt's lymphoma
	Nasopharyngeal carcinoma
	Some forms of Hodgkins disease
	Other non-Burkitt's lymphomas
Hepatitis B (and C) viruses	Hepatocellular carcinoma
Human papilloma virus	Cervical carcinoma
	Some head and neck cancers
Human T cell leukemia virus	Adult T cell leukemia
Kaposi's sarcoma-associated herpes virus (HHV-8)	Kaposi's sarcoma
	Body cavity lymphomas
	Castleman's disease

tries. HHV-8 appears to be widespread in Africa, but endemic KS is much more common in East and Central Africa. HHV-8 is associated with three neoplastic disorders: KS, primary effusion lymphoma (PEL) (also called body cavity-associated lymphoma), and diffuse Castleman's disease. KS is generally found in four forms: (*i*) sporadic (generally in elderly Mediterranean men); (*ii*) endemic (to certain areas of Africa); (*iii*) iatrogenic (in immunodepressed patients); and (*iv*) epidemic (associated with HIV infection and AIDS). KS is characterized by the proliferation of a spindle cell component, abundant, irregular angiogenesis, and an inflammatory infiltrate. Cells forming KS lesions are euploid. KS regression on cessation of immunosuppressive treatment in iatrogenic or sporadic KS indicates that KS is not a true sarcoma but a reactive granulation tissue. Epidemiologic studies strongly suggest that there is a common infectious agent involved in the pathogenesis of KS because HHV-8 sequences are found in lesions in all forms of KS. PEL is a rare form of AIDS-related B cell lymphoma characterized by malignant effusions in the pleural or abdominal cavity, Ig gene rearrangement, and lack of c-myc rearrangements. Most patients with PEL are dually infected with EBV and HHV-8, but occasional cases of EBV-negative/HHV-8-positive PEL have been reported. Most PEL lymphoma cells harbor multiple copies of HHV-8 as episomes. Diffuse Castleman's disease is an atypical lymphoproliferative disorder, and most cases in HIV-infected patients contain detectable HHV-8. HHV-8 has been found to encode several homologues of cellular genes, which may enable the virus to facilitate cell proliferation, and the orfK1 gene product is capable of transforming cells in culture. Thus, although HHV-8 is strongly associated with these tumors, the exact mechanism of viral transformation remains to be determined.

There are approximately 70 types of papillomaviruses that infect humans (HPVs), and several of these are strongly associated with malignancies of the human anogenital tract, and in particular cervical cancers. Newer evidence has been reported of the involvement of HPV in some head and neck cancers (HPV-11, -13, and -30). Although HPV-6 or HPV-11 are relatively common, they are only rarely associated with tumors. Thus, infections with these two types of HPV are benign and generally considered low risk for developing into cancers. In contrast, infection by several other types of papilloma viruses is considered as a high risk for malignant conversion (most often types 16, 18, 45, and 56, as well as 31, 33, and 35). In these HPV-associated tumors, the viral DNA is integrated within the genome of the tumor cell, and the known transforming genes of these viruses (E6 and E7) are transcribed. The mechanism by which the products of these viral genes promote malignant transformation involves binding to and altering the functions of cellular tumor suppressors (p53 and Rb). E6 promotes ubiquitination of p53, leading to its proteolytic degradation. E7 complexing to Rb results in the release of the transcription factor, E2F, which in turn activates transcription of genes involved in cell proliferation. Although E6 and E7 alone may be necessary for HPV malignant transformation, by themselves they do not appear to be sufficient, and subsequent genetic changes are required for frank malignancy.

What about other tumors in which a viral association has been implicated? It must be emphasized that the ability of a virus or one of its components to immortalize or transform primary cells in culture is not sufficient to convict a human cancer virus. For example, adenovirus 12 can form tumors in animal model systems, and the viral E1A and E1B gene products can transform certain cells in culture. Yet, adenoviruses are not implicated in human cancers based on lack of any epidemiologic association and absence of evidence that viral gene products are present in human tumors.

Recent evidence from several laboratories suggests that SV40-specific DNA sequences can be detected by PCR-based techniques in patients with mesotheliomas and other tumors (4, 9–12). However, this association remains controversial. SV40 was a contaminant of early poliovirus vaccines administered before 1964, and there is no epidemiologic evidence that polio vaccination led to a higher incidence of tumors, even in virus excreting vaccinees (13, 14). As with adenovirus, SV40 is able to induce tumors in animal models, and the viral T antigen gene product alone can immortalize certain cells in culture. However, SV40 viral-encoded antigens are highly immunogenic, and tumors are very difficult to induce in competent animal hosts. Thus, the jury is still out regarding SV40 because of the absence of data to implicate viral encoded T antigen, or other markers of stable viral association, in human tumors as opposed to normal tissues.

In their recent study, Laghi *et al.* (3) attempt to indict another polyoma virus. They provide data that indicates that JC viral DNA is present in malignant colorectal epithelial tissue. Using PCR-based techniques, they observed at least 10× more JC virus DNA in cancerous material than that detected in normal colonic tissue. JC virus is the presumed causative agent of an extremely rare, subacute, progressive demyelinating disease of the central nervous system called progressive multifocal leukoencephalopathy (PML) (15–17). PML occurs only in immunosuppressed individuals, generally older than 40 years, and is caused by the lytic replication of the virus in oligodendrocytes. The virus probably causes a subclinical persistent or latent infection in many healthy people, and viral DNA has been found in spleen, bone marrow, and cerebrospinal fluid (18, 19). The virus is often present in the urine of PML or other immunosuppressed individuals, suggesting that it is carried latently in the kidneys (20, 21). At least 70% of normal healthy adults have antibodies to JC virus, and primary infection occurs early in life (22–24). Not every immunosuppressed individual develops PML, and all of the factors leading to disease are not known. At least half of the PML cases also have Hodgkin's disease or chronic lymphocytic leukemia. PML has been reported in renal transplant patients undergoing immunosuppressive therapy, and as many as 4% of AIDS patients develop the disease. The JC virus T antigen is able to alter expression of several cell cycle regulators (25), and it as well as the T antigens of the related SV40 and human BK viruses induce tumors in a variety of tissues in transgenic mice (26–29). The BK virus was isolated from the urine of an immunosuppressed renal transplant patient (30). BK virus bears little antigenic cross-reactivity with JC virus and causes no known disease. Although BK virus DNA has been demonstrated to be present in human brain tumors (31), its role in causing these tumors remains to be investigated.

The new findings by Laghi *et al.* (3) are provocative and point to a possible new JC viral association with human cancer. The application of the systematic epidemiologic and mechanistic approaches summarized above will be needed to assess this possibility. In addition, such studies would have to account

for the lack of any known increase in colon cancer in immunocompromised patients. Thus, these authors present us with a smoking gun, but further investigation will be needed to convict this agent as a human cancer virus.

We thank Martine Aubert for help in preparing the manuscript.

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