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Viruses in Human Cancers

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Viruses may contribute to the development of human tumors by different mechanisms: indirectly by inducing immunosuppression or by modifying the host cell genome without persistence of viral DNA; directly by inducing oncoproteins or by altering the expression of host cell proteins at the site of viral DNA integration. Human cancers associated with papillomavirus, hepatitis B virus, Epstein-Barr virus, and human T cell leukemia-lymphoma virus infections are responsible for approximately 15 percent of the worldwide cancer incidence. Cancer of the cervix and hepatocellular carcinoma account for about 80 percent of virus-linked cancers. Because experimental and epidemiologic data imply a causative role for viruses, particularly in cervical and liver cancer, viruses must be thought of as the second most important risk factor for cancer development in humans, exceeded only by tobacco consumption.

VIRUSES CAN CONTRIBUTE TO THE DEVELOPMENT OF HUman tumors by a variety of mechanisms that range from genetic stimulation of host cell proliferation to virus-induced immunosuppression that permits the emergence of tumors not directly related to the suppressing virus (Table 1). A patient who is infected with human immunodeficiency virus (HIV) has a substantially increased risk for developing certain cancers, most notably Kaposi sarcomas and B cell lymphomas. These tumors appear to result from immunosuppression caused by HIV infection. Although mice transgenic for the HIV *tat* gene develop tumors similar to Kaposi sarcoma, specific genetic information for *tat* has not yet been found in human Kaposi sarcomas. Herpes simplex viruses (HSVs), on the other hand, have been suspected of contributing to some tumors, particularly anogenital and oral cancers (*1*), on the basis of seroepidemiological studies and reports on in vitro transformation of rodent cells by partially inactivated HSV preparations. Although these viruses are able to induce mutations in host cell DNA and to amplify specific intracellular DNA sequences under conditions of abortive infections (*2*), many recent studies failed to provide evidence for their involvement in human cancers. In this article I will therefore concentrate on those viruses for which tumor development appears to be the direct consequence of a specific infection and where trans or cis effects of viral genome persistence seem to contribute to the stimulation of cell proliferation.

Although members of at least three other groups of viruses exert cell-transforming properties, for example, polyomaviruses (BK, JC, and LPV), adenoviruses (particularly types 12 and 18), and poxviruses (molluscum contagiosum), none of them has yet been regularly documented to be present in human tumors. JC and BK virus genomic DNA in gliomas and insulinomas (*3*) occurs only in a fraction of tumor cells; thus these results are presently inconclusive.

Epstein-Barr virus (EBV), hepatitis B virus, several types of papillomaviruses, and HTLV-I and possibly -II (human T cell leukemia-lymphoma virus) are consistently linked to specific malignancies and will be discussed in greater detail (Table 2). None of these virus infections per se is sufficient to induce cancer. Long latency periods, often lasting several decades, the low number of infected individuals who eventually develop the particular type of cancer, monoclonality of the tumors, and in some instances interactions with chemical or physical factors in carcinogenesis (*4*) point to the requirement for additional modifications in cancer development

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after the viral infection. Nevertheless, because virus-linked human cancers are responsible for 15% of the total cancer incidence (*4*), specific viruses are the second most important risk factor for cancer development in humans, exceeded only by tobacco consumption.

Epstein-Barr Virus and Human Tumors

EBV has been linked to at least four different types of human malignant tumors; its role in the African form of Burkitt's lymphoma and in nasopharyngeal cancer is particularly well documented. Substantial evidence also links this virus infection to many B cell lymphomas in immunosuppressed individuals, particularly after organ transplantation or HIV infections. It has also been postulated that this virus contributes to Hodgkin's lymphoma, where a clonal form of viral DNA occurs in up to 40% of patients within Hodgkin-specific Reed-Sternberg cells (*5*). Sporadic demonstrations of EBV DNA in other tumors remain unconfirmed.

EBV is a ubiquitous viral infection and is present in up to 100% of the population. Even in affluent societies with high standards of hygiene, infections may reach 90% of the population late in adolescence or in young adults. Early infections, usually within the first year of life, are common in developing countries, whereas infections during adolescence are far more common in industrialized countries, accompanied in part by the typical symptoms of infectious mononucleosis (*6*). EBV persists in the infected host throughout life, and virus shedding is regularly observed in salivary gland excretions. This continuous production of infectious particles most likely accounts for the high rate of virus transmission. Another recently discovered member of the herpesvirus group, HHV-6, can also be transmitted through saliva but has not been linked to specific human tumors.

It was initially suggested that EBV might be a causal agent in Burkitt's lymphoma when the virus was isolated from Burkitt's lymphoma cells kept in tissue culture (*7*) and when seroepidemiological studies revealed high antibody titers in Burkitt's lymphoma patients in comparison to age-matched controls (*8*). EBV DNA was detected in "virus-free" Burkitt's lymphoma cell lines and in biopsy material obtained from such tumors as well as from nasopharyngeal cancer cells (*9*). The demonstration of virus-specific antigens in these cancers, EBV-induced immortalization of B cell lymphocytes, the induction of a lymphoma-like disease in New World primates by EBV (*10*), and prospective seroepidemiological studies in Uganda (*11*) further supported the notion that EBV could cause these cancers. Yet, 27 years after its discovery, the mechanism by which EBV contributes to the development of Burkitt's lymphoma is still obscure, in part because a small percentage (about 5%) of Burkitt's lymphomas are EBV-negative in the endemic African regions and the majority of the sporadic Burkitt's lymphoma cases occurring worldwide are free of EBV.

Table 1. Viral functions potentially involved in oncogenesis.

Function	Viruses					
	EBV	HBV	HPV	HTLV	HSV	HIV
Stimulation of cell proliferation by viral oncogene (trans effect)	+	+?	+	+	−	−
Promoter insertion (cis effect)	−?	+?	−?	−?	−	−
Mutagenic effects on host cell DNA	−?	?	+?	−?	+	−
Nonspecific induction of host cell proliferation (cell regeneration, inflammatory reaction)	−	+	−	−	+	−
Immunosuppression	−	−	−	−?	−	+

Table 2. Viruses linked to human tumors.

Virus type	Benign proliferations	Malignant tumor
EBV	Hairy leukoplakia Infectious mononucleosis	Burkitt's lymphoma Nasopharyngeal cancer B-lymphomas in immunosuppressed individuals
HBV	Focal liver hyperplasia	Hepatocellular carcinoma
HPV types 5, 8, 14, 17, 20	Cutaneous plaques and papillomas in patients with EV	Skin carcinomas usually at sun-exposed sites in renal allograft and in EV patients
HPV types 16, 18, 31*, 33, 35*, 39*, 45*, 51*, 52, 56*, 58*, 59*, 61*	Cervical intraepithelial neoplasia; vulvar, penile, and perianal intraepithelial neoplasias	Cervical cancer Vulvar cancer Penile cancer Perianal and anal cancer
HPV types 6, 11	Condyloma acuminatum	Verrucous carcinoma of vulva and penis, Buschke-Löwenstein tumors
HTLV-I	Smouldering leukemia	Adult T cell leukemia

*Only rarely found.

The most consistent finding in Burkitt's lymphomas, irrespective of their EBV status, is a chromosomal translocation involving immunoglobulin genes, usually on chromosome 14 or less frequently on chromosome 2 or 22, and sequences within or adjacent to the c-*myc* locus on chromosome 8. These translocations result in c-*myc* deregulation, increased transcription of the modified allele, and autosuppression of the unaffected c-*myc* locus. The consistency of this rearrangement, combined with the observation that mice transgenic for the rearranged c-*myc* locus develop B cell hyperplasia and monoclonal B cell lymphomas (*12*), implicates these events in the development of Burkitt's lymphomas.

On the other hand, EBV infection of B lymphocytes leads to rapid lymphoblast transformation accompanied by the expression of new surface markers and immortalization of these cells when kept in tissue culture. Although most cell lines derived from Burkitt's lymphomas will cause tumors in nude mice and form colonies of semisolid media, EBV-immortalized lymphoblastoid cells regress after an initial proliferative period in nude mice and are thus nontumorigenic and fail to grow in colonies in soft agar. Although the transforming genes of EBV have not yet been unambiguously identified, EBNA-1, EBNA-2, and the latent membrane protein (LMP) are candidates. EBNA-2 and LMP expression is suppressed in Burkitt's lymphoma cells (*13*). This may be related to the state of differentiation of Burkitt's lymphoma cells (*14*), which represent an early stage in the B cell lineage. Thus, it is presently not possible to attribute the development of Burkitt's lymphomas either to c-*myc* deregulation or to EBV infections. Conflicting data have been reported after transfection of EBV-immortalized lymphoblasts with a rearranged c-*myc* construct: Lombardi and co-workers (*15*) observed tumorigenicity of the transfected lymphoblasts, whereas Hotchin and co-workers (*16*) failed to find tumorigenicity under similar conditions. Hybrid cells resulting from fusions between Burkitt's lymphoma cells and EBV-transformed lymphoblasts derived from the same donor express exclusively the rearranged c-*myc* and down-regulate the expression of EBNA-1 and EBNA-2 from Burkitt's lymphoma cells (*17*). Yet all hybrid clones remain nontumorigenic in nude mice when observed for prolonged periods of

time, indicating that the deregulated c-*myc* expression in combination with expression of EBV latent genes is not sufficient for tumorigenicity under these conditions.

In contrast to the results with tumor-forming Burkitt's lymphoma cells, after inoculation of EBV-immortalized lymphoblasts or hybrid cells a tumor is formed but it undergoes central necrosis and regression within 2 to 4 weeks (*17, 18*). During the first 2 weeks, cell proliferation in nude mice proceeds in the Burkitt's lymphoma tumors as well as in the EBV-immortalized lymphoblast and hybrid "tumors" approximately at the same rate. Even after appearance of necrotic centers, adjacent cell layers reveal mitotic figures (Fig. 1). This pattern suggests that the difference in tumorigenicity between EBV-immortalized lymphocytes and Burkitt's lymphoma cells is probably not related to the proliferative capacity of the cells but that it may be the result of differences in either the production or the reactivity to tumor necrosis factor (TNF)–like molecules produced by the lymphoblasts. This difference may also account for the failure to clone EBV-immortalized lymphoblasts in agar. Lymphoblasts and other cell types can produce TNF, and in tumor cells this production correlates with reduced tumorigenicity (*19*). Therefore, the chromosomal translocations in Burkitt's lymphomas occurring at an early stage of B cell differentiation may disrupt at least one additional gene, probably physically linked to the c-*myc* locus, which regulates the synthesis or the reactivity to the proposed TNF-like factor.

Burkitt's lymphomas could thus develop as a consequence of differentiation arrest due to the translocation event and subsequent growth stimulation resulting from c-*myc* deregulation and persistent EBV infection. The host defense mechanisms could be undermined by the observed but yet mechanistically unexplained suppression of EBV membrane antigen expression and by blocking apoptotic responses potentially mediated by an endogenous TNF-like factor. EBV-negative Burkitt's lymphomas could result from similar events that also involve an additional yet unidentified growth-stimulatory function.

The contribution of EBV infections to nasopharyngeal cancers is even less characterized. High antibody titers against EBV-specific antigens, the predictive value of immunoglobulin A titers to EBV antigens for tumor development (*20*), the regular presence of viral DNA in the tumor cells (*9, 21*), and the continuous expression of this DNA point to a role for this virus in the development of this cancer. However, the different geographic distribution of endemic regions and differences in age incidence implicate other cofactors in the etiology of these tumors. The role of EBV in the induction of B cell lymphomas in immunosuppressed patients and in patients with Hodgkin's disease awaits further clarification. Although EBV DNA occurs in Reed-Sternberg cells of a certain percentage of patients with Hodgkin's disease (*5*) and EBV antibody titers are elevated in these patient groups, the percentage of EBV-negative Hodgkin patients corresponded exactly to that of age-matched control groups (*22*). Future experiments are needed to elucidate this situation. Although the pathogenesis of none of the EBV-linked malignant tumors is as yet understood, EBV clearly emerges as the first identified human tumor virus that contributes to important proliferative disorders in humans.

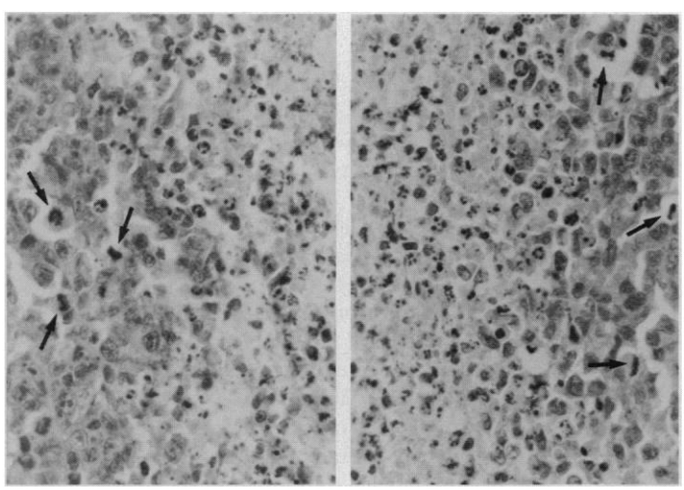

Fig. 1. Regressing nude mouse tumor originating from a Burkitt lymphoma-lymphoblastoid cell line hybrid clone. Note the mitotic figures (arrows) in close proximity to the necrotic plaque.

HTLV-I in Adult T Cell Leukemia

The discovery of retrovirus in human tumors (*23*) was the turning point in a long series of attempts to identify such viruses in humans that had been spurred by the well-established tumorigenicity of retroviruses in animals. HTLV-I was isolated with the use of interleukin-2 (IL-2)–stimulated T lymphocytes and shortly thereafter was linked to an endemically clustered form of leukemia-lymphoma [adult T cell leukemia-lymphoma (ATLL)] prevalent in coastal areas of southern Japan, in the Caribbean, and in regions of central Africa (*24*). The virus is transmitted vertically but also horizontally by breast feeding, sexual intercourse, blood transfusions, and needle sharing (*25*).

The latency period between primary infection and leukemia outbreak is several decades (*4*). This fact, together with the monoclonal integration pattern of HTLV-I DNA in ATLL cells, shows that virus infection per se is not sufficient for tumor development. HTLV-I antibodies are present in more than 1 million persons in Japan, but there is an annual incidence of about 500 ATLL cases in that country (*26*). Thus, 1 out of 25 to 30 infected individuals eventually develops ATLL. Nevertheless, HTLV-I DNA is regularly demonstrated in the leukemic cells. There is a monoclonal pattern of viral integration sites, the patients are positive for HTLV-I antibody, and there is a seroepidemiological pattern with a close correlation between areas of high prevalence of HTLV-I antibodies and the incidence of ATLL (*27*). Moreover, laboratory studies demonstrate the capacity of the virus to immortalize human $CD4^+$ T lymphocytes that express markers for activated T cells such as the IL-2 receptor and HLA-DR antigen (*24, 28*). HTLV-I induces the expression of IL-2 and IL-2 receptor genes in infected T cells, therefore providing an autocrine loop for T cell proliferation (*28*).

In addition to genes common to all retroviruses, the HTLV-I genome contains an approximately 1.6-kb 3′ terminal region, *X*, that is related to the T lymphocyte–immortalizing phenotype of the virus (*29*). This region codes for at least two trans-acting regulatory proteins, the 40-kD protein product of *tax*, a transcriptional regulator, and the product of *rex*, a 27-kD phosphoprotein that is a posttranscriptional regulator (*30*). Mice transgenic for *tax* develop multiple mesenchymal proliferative disorders at about 3 months of age with nodular growths in the tails, ears, extremities, and around the face, with some tumor clustering around sites of repeated trauma and at the base of the cranium (usually in pregnant mice) (*31*).

These data imply that HTLV-I is critical in the development and progression of ATLL. Yet questions still remain: in spite of a rapid expression of persisting viral DNA in leukemic cells when explanted into tissue culture (*32*), this viral activity is difficult to demonstrate in fresh leukemic cells. The mode of this suppression is still unknown. The proliferative capacity of peripheral leukemic cells is not well characterized. Viral gene expression and leukemic cell

proliferation may be correlated in other lymphatic organs. Consistent chromosomal abnormalities, increasingly found in other human tumors, have not been conclusively demonstrated in ATLL. Most common changes involve trisomies of chromosome 7, the loss of X chromosomes in females, and occasional translocations in chromosome 14 (*33*).

As in other human pathogenic tumorvirus systems, ATLL development most likely involves interdependent genomic modifications of the persistently infected host cell, most of which are presently unknown. Yet, the identification of this retrovirus as a prime factor in ATLL development not only provided a substantial boost to retroviral oncology but also paved the way for the isolation of additional human pathogenic retroviruses, such as HTLV-II and HIV.

Hepatitis B Virus and Hepatocellular Carcinoma

Epidemiological observations pointed initially to a link between chronic hepatitis B virus (HBV) infections and hepatocellular carcinoma (HCC) (*34*). In addition to there being a coincidence of areas with a high prevalence of chronic HBV and HCC, a wide range of case control and prospective cohort studies demonstrate that chronic HBV infections are highly significant risk factors for HCC development. This is particularly evident in high-risk areas for HCC, such as Taiwan, Senegal, South Africa, Hong Kong, the People's Republic of China, and the Philippines (*35*). It is less pronounced in low-risk areas, for example, the United States, England, and Wales (*36*).

The appearance of HCC after persistent HBV infection, usually after several decades of chronic liver disease and continual replacement of damaged or destroyed hepatocytes by regeneration accompanied by frequent incidences of liver cirrhosis, led to speculations suggesting that HCC development is the consequence of nonspecific chronic regenerative events (*37*). Although continuous liver cell regeneration may allow an accumulation of mutational events that eventually results in the emergence of an HCC clone, it also appears that HBV genome persistence is critical. This is based on several lines of observations: (i) HBV DNA persists in the majority of tumors originating in individuals from high-risk areas (*38*). (ii) Although commonly rearranged, the integration pattern of HBV points to a monoclonal origin of viral DNA. At least parts of the specific open reading frame *X* are preserved in these tumors (*39*). (iii) *X* gene expression as well as expression of another open reading frame (*pre-S*) results in transcriptional activation in trans (*40*). (iv) Multifocal nodular hyperplastic liver disease has been observed in mice transgenic for a subgenomic fragment of HBV containing the coding information for the three envelope polypeptides containing hepatitis B virus surface (HBs) antigen (*41*). (v) Expression of a cloned *X* gene under the control of a strong foreign promoter induced NIH 3T3 cells to form tumors in nude mice (*42*). (vi) Transfection of fetal mouse hepatocytes carrying SV40 T antigen under the control of the metallothionein promoter with HBV DNA resulted in the establishment of malignant clones containing rearranged HBV DNA (*43*). (vii) Liver cancer developed in mice transgenic for the *X* gene (*44*).

Another set of observations has been obtained from animal experiments with closely related hepadnaviruses. Such agents have been isolated from woodchucks, domestic ducks, ground squirrels, geese, and grey herons (*45*). The woodchuck hepatitis virus (WHV) induces liver cancer more than 1 year after inoculation into newborn woodchucks (*46*). In these animals no cirrhosis develops and HCCs arise in a background of minimal hepatitis with a small degree of periportal lymphatic infiltration. The viral DNA is rearranged and reveals a monoclonal integration pattern.

The interpretation of these data is still very difficult. They do not yet rule out the possibility that regenerative events are the sole driving force for HCC development. It is, however, more likely that specific viral functions in cis or in trans are important for initiation and maintenance of the proliferative changes. Cis effects have been postulated to occur on the basis of rearrangements and enhanced expression of c-*myc*, in *erb*-A or steroid receptor genes in HCCs after integration of hepadnavirus DNA often in the proximity of the respective loci (*47*). On the other hand, rearrangement of *X*- or *pre-S* coding sequences may modify the function and lifetime of chimeric transcripts and thus stimulate liver cell proliferation (*48*).

Specific modifications in the short arm of chromosome 11 and deletions in the long arm of chromosome 13 occur in HCC (*49*). This result points to the existence of tumor-suppressing genes at these locations. These negative regulator elements may normally prevent HCC. Moreover, point mutations in the p53 tumor suppressor gene have been recorded (*50*). They appear to emerge preferentially in areas with a high rate of food poisoning by aflatoxins, thus suggesting an interaction between HBV infection and chemical carcinogens. HCCs that are negative for HBV may be in part related to an RNA viral infection, hepatitis C virus, which also leads to chronic persistent infections with liver injury and liver cell regenerations (*51*). Ongoing HBV vaccination studies in West Africa and Southeast Asia should further clarify the role of HBV in the incidence of HCC.

Papillomaviruses and Anogenital Cancer

Papillomaviruses (PV) are a group of epitheliotropic viruses found in a wide variety of species (*52*). They cause benign epithelial proliferations but have also been linked to cancers in animals and humans. The cottontail rabbit papillomavirus (CRPV) induces papillomas that frequently convert into squamous cell carcinomas. More than 60 humanpathogenic papillomavirus (HPV) genotypes have been isolated thus far (*53*). A large number of genotypes has been isolated from a rare hereditary condition, epidermodysplasia verruciformis (EV) (*54*), which predisposes patients to these infections. Cancers arising in EV, commonly in papillomas that have been exposed to the sun, most frequently contain HPV-5 DNA, and occasionally also other types of HPV (Table 2). These tumors are an example of cooperative effects of specific virus infections and other environmental carcinogens.

A substantial number of HPV genotypes that infect the anogenital tract have been isolated and characterized (Table 2). These viruses are particularly suited for the analysis of pathogenic aspects of viruses in carcinogenesis: specific HPV types are found in anogenital cancers; these cancer types constitute about 10% of the cancers occurring worldwide (*4, 55*); cell lines derived from individuals with cancer of the cervix and containing HPV are available; and HPV can immortalize human keratinocytes in vitro.

Some types of HPV infection of the anogenitalia present a high risk for malignant conversions (for example, HPV 16 and HPV 18). Infections by other types (HPV 6 and HPV 11) rarely result in invasive tumors and are considered low-risk.

These high-risk types of HPV are thought to be causally involved in the pathogenesis of anogenital cancer, particularly in cancer of the cervix (*56, 57*). The evidence includes the following: (i) Viral DNA is found in close to 90% of such tumors (most frequently DNA of HPV 16). (ii) Most of the virus-positive tumors contain integrated viral DNA. The viral circular episome is disrupted within a specific region (E2 open reading frame) for integration. No consistent

pattern has been observed for intrachromosomal localizations. A small percentage of malignant tumors harbors nonintegrated copies of viral DNA (*58*). (iii) The vast majority, if not all, of HPV-positive cancer biopsies and all HPV-containing cell lines derived from individuals with cervical cancer reveal specific transcripts originating uniformly from two specific open reading frames (E6 and E7) of the persisting HPV DNA (*59*).

The E6 and E7 genes of high-risk types of HPV (but not of low-risk types) immortalize human foreskin, cervical keratinocytes (*60*), or epithelial breast cells after in vitro transfection in tissue culture (*61*). In organotypic cultures these immortalized cells share growth characteristics with intraepithelial neoplasias (*62*). Although HPV E6-E7 immortalized human cells are initially nontumorigenic in nude mice, long-time in vitro cultivation may lead to malignant clones (*63*). This result demonstrates that these HPV infections alone can induce malignant growth, provided a sufficient number of cell generations permits the manifestation of additional spontaneous or virus-gene–induced modifications. Chromosomal instability of immortalized cells has been regularly observed in such cultures and may be the source of the additional progressive changes. In fact, aneuploidy has been described in HPV 16– or 18–positive clinical lesions but is absent in low-risk infections (*64*).

There are other differences between high-risk and low-risk infections. Specific cellular proteins bind efficiently to the HPV E6-E7 oncoproteins of high-risk types of HPV (*65*). The E7 oncoprotein binds the protein of the retinoblastoma suspectibility gene (Rb), whereas E6 binds to the p53 protein, which is a tumor suppressor gene product (*66*). The E6 binding of p53 promotes the degradation of p53 (*67*).

Rb and p53 appear to play as yet poorly defined roles in the regulation of cellular DNA replication and the mitotic cycle. Interference with their function may lead to deregulation of the cell cycle and to chromosomal instability and aneuploidy as regularly observed in individuals with high-risk HPV infections (*62, 68*). E6 or E7 proteins from persons with low-risk HPV infections (HPV 6 or 11) appear to bind either less actively or not at all to these host cell proteins (*65*); they also fail to induce chromosomal changes. Thus, the interaction of high-risk HPV E6-E7 gene products with cellular Rb and p53 protein may represent an endogenous progression factor and may be important for the progression of premalignant and malignant stages of carcinogenesis as the consequence of the induction of mutations and chromosomal instability; p53 mutations have only been noted in HPV-negative cervical carcinoma cell lines but not in HPV 16– or 18–positive lines (*69*).

In addition to these endogenous host cell DNA modifications, the contribution of mutagenic exogenous factors (for example, smoking or other infections with mutagenic potential) to cancer development probably requires reassessment. Part of the difference in the carcinogenic potential between low-risk and high-risk HPV infections may be due to the dependence of the low-risk types on exogenous factors for malignant conversion whereas in the high-risk types the endogenous events could largely cause premalignant and malignant progression.

The important role of E6-E7 proteins in cell proliferation and tumor development is underlined by experiments showing that hormone-dependent expression of E6-E7 antisense constructs reduces cell growth and leads to nontumorigenicity of HPV-containing cervical carcinoma cells, as well as rodent cells (*70*). In addition, dexamethasone-inducible overexpression of E6-E7 genes in certain cervical carcinoma cell lines and inhibition of these genes in another line result in corresponding changes in tissue culture growth and tumorigenicity (*71*). Thus, E6-E7 genes may not only participate in the initiation but also may maintain the proliferative and malignant phenotype.

However, viral E6-E7 gene expression is necessary but not sufficient for the development of malignant growth:

1) Cancer of the cervix develops from precursor lesions [high-grade cervical intraepithelial neoplasias (CIN)] that contain the same high-risk HPV types as the respective cancers that emerge at these sites after latency periods of years or decades (*4*).

2) In vitro E6-E7 immortalized human cells are not malignant in early passages in spite of E6-E7 gene activity (*60*); however, additional transfection with v-*ras* oncogenes renders them malignant (*72*).

3) Fusion of HPV-positive cervical carcinoma cells with normal human cells results in nonmalignant hybrids despite the presence of E6-E7 gene expression in these hybrids (*73*).

Observations 2 and 3 can be explained in part by the fact that inoculation of immortalized foreskin or nontumorigenic hybrid cells into nude mice results in a reduction of E6-E7 gene expression (*73, 74*). This result contrasts with the reaction of malignant cells, which continue to actively transcribe E6-E7 genes. Thus, in malignant cells, there is a failure of an intracellular regulation of viral gene expression as postulated previously (*14, 57, 75*). This regulation appears to be triggered after explantation of the cells into the animal.

It has been postulated that transforming growth factor (TGFβ) and epidermal growth factor (EGF) are potential humoral factors (*57*) that may suppress HPV expression in nonmalignant cells. Both reduce HPV transcription in immortalized cells and fail to act on malignant cells (*76*). TGFβ also exerts a growth-inhibitory effect on nonmalignant cell lines. The molecular mechanism of this inhibition is not understood.

The selective down-regulation of HPV E6-E7 expression in HPV-positive nontumorigenic hybrid cells or in immortalized keratinocytes can also be seen in experiments with 5-azacytidine, a potent demethylating compound (77). Malignant cells fail to respond to this treatment. In nonmalignant cells, triggering of the down-regulation of HPV expression in tissue culture or in the animal may depend therefore on demethylation of specific host cell genes.

A suppressive effect of host cell genes on the viral long regulatory region (LRR) that governs E6-E7 gene expression can be demonstrated in nonmalignant cells by other experimental approaches: constitutive expression of indicator genes (chloramphenicol acetyltransferase, CAT) under the control of the HPV 18LRR in cervical carcinoma cells obtained under nonselective conditions is extinguished after they are fused with nonmalignant cells (*78*). Suppression occurs by inhibition of initiation of transcription and is inactivated after 4 hours of cycloheximide treatment, pointing to a monogenic suppression by a short-lived protein.

In this system, down-regulation of the HPV promoter-driven CAT construct is not accompanied by a reduced expression of the endogenous HPV sequences in the cervical carcinoma cells. This difference in the regulation of exogenous HPV promoters most likely originates from cis effects of flanking host cell sequences at the integration site of endogenous HPV DNA within the host cell genome (*71, 79*).

The location of viral DNA integration seems to be important in the deregulation of HPV gene expression, as already supported by the higher frequency of integrational events in cancer cells than in premalignant lesions (*58, 80*). Because these integrational events regularly disrupt the E2 open reading frame, abolishing its transactivating and repressing properties (*79*), intragenomic dysregulations may favor enhanced expression of E6-E7 genes. However, depending on the site of viral DNA integration into the host cell genome, expression of these genes may also be suppressed (*58, 81*). It is therefore likely that differences in E6-E7 gene regulation in nonmalignant cells in tissue culture and after inoculation into nude

mice are controlled by the localization of viral DNA integration but may also be influenced by specific host cell methylation and demethylation of genes that suppress the persisting viral DNA.

In addition to methylation of host cell genes engaged in the regulation of viral DNA, direct methylation of persisting viral DNA may contribute to modification in viral gene expression. In HeLa-human fibroblast hybrids, the persisting HPV 18 DNA is more intensively methylated than in malignant segregants obtained from the same hybrid clone (*77*). The latter still contain more methylated HPV sequences than the parental HeLa cells. Thus, specific modes of methylation and demethylation may be one mechanism by which the postulated cellular interfering factors (*57, 75*) may operate. De novo methylation of specific sites in tissue culture has been reported in other systems (*82*).

At present there exists no support for the view that regulation of viral gene expression in nonmalignant cells is due to the interaction of E6 and E7 oncoproteins with p53 and Rb proteins. At least p53 appears to be transcribed in nonmalignant and malignant cells (*83*), although the amounts of p53 in many cervical carcinoma cell lines and in HPV-immortalized lines are generally low (*67*). Suppression of HPV transcription occurs by inhibition of initiation of transcription (*77, 78*) and chromosome 11, which harbors neither the Rb nor the p53 gene, is important in suppressing the malignant phenotype of cervical carcinoma cells (*84*). The elucidation of this regulatory mechanism and the identification of the pertinent genes will be of substantial interest and potential and may also aid in understanding the mechanisms of latency of other persisting viruses.

Conclusions

It is presently difficult to assess the total contribution of virus infections to cancer development in humans. Immunosuppression as a consequence of HIV infections clearly increases the risk for specific tumors (most notably Kaposi sarcomas and B cell lymphomas) as an indirect result of a viral infection.

More direct virus–host cell interactions are noted in the development of other human tumors: among those, cancer of the cervix and HCC are the most prevalent tumors linked to specific virus infections. They account for approximately 80% of all presently virus-linked human cancers (*4*). The expression of specific viral oncoproteins appears to be required for the malignant phenotype of HPV-positive anogenital cancers. The contribution of HBV gene functions to the development of HCC is less clear. Functional modifications of host cell genes in the vicinity of the viral DNA integration site may in this case influence the progression to malignancy.

HTLV-I is a retrovirus infection linked to adult T cell leukemia with substantial experimental and epidemiologic support for an etiologic relationship.

The association of EBV with Burkitt's lymphoma and nasopharyngeal cancer was noted in the 1960s. EBV represents a B cell–immortalizing virus and induces lymphoproliferative disease in certain species of New World nonhuman primates. Yet, molecular mechanisms of EBV interaction with human tumors are presently poorly understood.

Infection with none of the viruses linked to human cancers leads directly to cancer development. Additional modifications of host cell DNA result in the stepwise progression to a malignant phenotype of the infected cell. Yet there exists good reason to assume that the elimination of the respective viruses as risk factors would greatly reduce the incidence of those cancers presently linked to these infections.

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Recombinant Toxins for Cancer Treatment

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Recombinant toxins target cell surface receptors and antigens on tumor cells. They kill by mechanisms different from conventional chemotherapy, so that cross resistance to conventional chemotherapeutic agents should not be a problem. Furthermore, they are not mutagens and should not induce secondary malignancies or accelerate progression of benign malignancies. They can be mass-produced cheaply in bacteria as homogeneous proteins. Either growth factor–toxin fusions or antibody-toxin fusions can be chosen, depending on the cellular target.

RECOMBINANT TOXINS ARE HYBRID CYTOTOXIC PROTEINS made by recombinant DNA technology that are designed to selectively kill cancer cells. The cell-targeting moiety can be a growth factor or a single chain, antigen-binding protein. The toxic moiety is a portion of a bacterial or plant toxin. Immunotoxins are similar in concept but are composed of antibodies chemically linked to toxins.

More than 30 years ago chemotherapeutic drugs began to be used to treat cancer as a supplement to surgery and radiation therapy. Now that several decades have passed, it is clear that the current generation of chemotherapeutic drugs can achieve cures of certain leukemias and lymphomas and, in the adjuvant setting, prolong the lives of patients with breast cancer, ovarian cancer, and several other types of cancer. Because chemotherapy is not a cure for the common types of cancer in adults, new therapies must be developed.

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One approach is to target a cytotoxic agent to the cancer cell (*1*). To accomplish this, the cytotoxic agent is attached to an antibody or a growth factor that preferentially binds to cancer cells. The targets for this type of therapy can be growth factor receptors, differentiation antigens, or other less characterized cell surface antigens. It is now established that many cancers overproduce growth factor receptors that can function as oncogenes and promote the growth of the cancer cells (*2–4*). For example, the epidermal growth factor receptor is present in large amounts (up to 3×10^6 receptors per cell) in many squamous cell and epidermoid carcinomas, glioblastomas, and some metastatic ovarian and bladder cancers (*5–7*). Normal cells contain as many as 3×10^5 receptors per cell (*8*). The interleukin-2 (IL-2) receptor is present in substantial numbers on the cells of patients with adult T cell leukemia (ATL; 3×10^4 receptors per cell) and in lower numbers in various other lymphoid malignancies (*9*).

Differentiation antigens that occur on normal cells such as B lymphocytes are often also present on tumor cells such as B cell lymphomas. Because such antigens are not present on the stem cells