Viruses in human cancers*

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Infections have been recently identified as important etiologic factors for an increasing percentage of human cancers. Between 15 and 20% of the global burden of human cancers have been linked to viral, bacterial and parasitic infections. Viruses play a major role, since two types of infections, anogenital papillomavirus types and Hepatitis B virus account for about 15% of cancers in females and slightly less than 10% of cancers in males. This review summarizes the criteria used for the identification of carcinogenic infectious agents, analyses some of the known mechanistic contributions of viruses to carcinogenesis, presents some host and host cell responses to these infections, and discusses prospects for the prevention and therapy of virus-linked cancers.

PRESENTLY between 15 and 20% of the global cancer incidence can be etiologically linked to specific infections. Bacteria (*Helicobacter pylori*) and helminths (Schistosoma, Opisthorchis, Clonorchis) contribute to the development of bladder and rectal cancers and to cholangiocarcinomas. Viruses, however, are the main cancer risk factors. Hepatitis B and C virus infections are involved in hepatocellular carcinomas. Specific types of papillomaviruses cause one major human cancer, cancer of the cervix and play an additional role in a number of other anogenital, but also in some oropharyngeal and cutaneous cancers. Epstein–Barr virus, known as a human tumor-virus since 1964, human herpes-virus types 8 (HHV-8), and human T-lymphotropic retrovirus type 1 (HTLV-1) have also been identified as human tumor-viruses.

There still remain some tumor types as candidates for a possible infectious aetiology: this includes lymphomas and leukemias, but also some epithelial tumors. Human tumor-viruses are commonly ubiquitous. A low rate of infected individuals eventually develops the respective form of cancer. Malignant conversions, usually the consequence of additional genetic modifications in latently infected cells, occur under conditions of severe immunosuppression. It is exceedingly difficult to reveal the existence of as yet unknown tumor-viruses by epidemiological studies if these are ubiquitous. Their discovery will depend on sensitive molecular biological or immunological approaches. In addition, the possible transmission of animal tumor-viruses to humans which may only be able to express transforming genes in human cells, has only been scarcely investigated and cannot be ruled out.

Criteria to link a virus infection to the etiology of a human tumor

The presence of viral DNA within human tumor biopsies may serve as a hint of an etiological relationship. The same is true for seroepidemiological studies revealing elevated antibody titers against a specific virus. Table 1 lists some of the methods for the discovery of the role of human viruses in cancer induction.

Characteristic features in the development of virus-linked tumors add to the difficulties to identify the causative agent. These cancers commonly arise only after long latency periods of several decades between primary infection and development of the respective neoplasm. The tumors are clonal, their monoclonality can frequently be deduced from the integration pattern of viral DNA. Most of the infected individuals either clear the infection by immunological interference or harbor viral DNA for lifetime within specific cells without symptoms.

Viral DNA frequently persists in subgenomic fragments in virus-positive tumors. These tumors are consequently unable to give rise to infectious progeny. Therefore, Koch’s postulates to causally imply a bacterial infection in a specific disease cannot be applied to tumor-viruses. They were based on the isolation of the infectious agent, *in vitro* propagation, the re-inoculation into a susceptible animal host and the induction of symptoms analogous to those observed in the diseased patient.

Attempts have been made to account for these difficulties and to define new criteria linking virus infections to human cancer, most frequently involving epidemiological and seroepidemiological data. The different modes of virus contributions to cell transformation, including their

<table>
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<th>Table 1. Methods used for the initial discovery of human tumor-viruses and early data relating to their carcinogenic potential</th>
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<td>Lymphomas and Epstein–Barr virus</td>
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<td>Adult T-cell leukemia</td>
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<td>Kaposi sarcoma and HHV-8</td>
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Vaccinations will aid in case of hepatitis B and papillomavirus infections.

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role as direct or indirect carcinogens render such efforts rather difficult.

The following four criteria\(^3\,^4\) can be only considered as valid for those tumor-virus infections that permit the identification of a trans- or cis-acting viral gene or DNA-fragment and exclude all indirect contributions to carcinogenicity, as for instance by continuing immunosuppression (e.g. HIV infections). (i) Epidemiological plausibility and evidence that a virus infection represents a risk factor for the development of a specific tumor; (ii) Regular presence and persistence of the nucleic acid of the respective agent in cells of the specific tumor; (iii) Stimulation of cell proliferation upon transfection of the respective genome or parts there from in in corresponding tissue culture cells; (iv) Demonstration that the induction of proliferation and the malignant phenotype of specific tumor cells depend on functions exerted by the persisting nucleic acid of the respective agent.

Particularly the last point represents the most stringent criterion to link an infection to tumors and to separate it from co-factors.

**Mechanisms of virus-mediated cell transformation**

Several human tumor-viruses induce continuous proliferation (immortalization) of specific human tissue culture cells. Immortalization uniformly precedes malignant conversion which cannot be achieved in a single step. We define here malignant growth as invasive proliferation after heterografting cells into immunocompromised animals. During the past few years it became increasingly clear that individual steps resulting in immortalization and eventually in a malignant phenotype include modifications of specific cellular genes, part of them engaged in the control of the persisting viral genome. Here some known functions of viral oncogenes will be discussed. Table 2 lists human tumor-viruses and other human virus infections, the latter are carcinogenic in experimental animal systems without a similar role in humans.

Prospective epidemiological studies underline the role of hepatitis B and C viruses in the etiology of hepatocellular carcinomas. Yet, these viruses are unable to immortalize human cells in tissue culture. In spite of the definition of trans-activating functions of specific HBV and HBC proteins (see below), their contribution to malignant conversion is presently unknown.

Similarly, little is known of viral functions contributing to the malignant conversion of lesions associated with papillomavirus types (HPV 5, 8, 14, 17, 20 and a few others) in epidermodysplasia verruciformis (EV) patients. In contrast to anogenital malignant tumors, carcinomas in EV patients only exceptionally seem to contain integrated viral DNA\(^5\). The preservation of E6 and E7 genes under these conditions may suggest a similar important role as demonstrated for high risk anogenital HPV infections. Very recent data suggest that the E6 protein of some of these viruses blocks apoptosis after exposure to DNA damage, e.g. by ultraviolet (UV) light\(^6\). In addition, the promoter region is activated by UV exposure\(^7\). Since squamous cell carcinomas of the skin most commonly arise on sun-exposed sites, some of these viruses may contribute to malignant growth by an indirect mechanism.

The human polyomavirus types BK and JC, as well as various types of human pathogenic adenoviruses have not been consistently found in human tumors, they are however carcinogenic for newborn rodents. Under specific circumstances they are able to immortalize human cells, usually very inefficiently.

Epstein-Barr virus (EBV), human papillomaviruses (HPV), human T-lymphotropes virus (HTLV)-1, and human herpes-virus (HHV)-8 possess defined oncogenes that stimulate proliferation of specific human cells. Although trans-activating properties have been defined for the HBV X and pre-S antigens\(^8\,-\,^10\) and mice transgenic for these genes and those transgenic for the HBC core antigen develop hepatocellular cancers\(^11\), it is presently unknown whether and to which extent the same genes contribute to human liver cancers.

Epstein-Barr virus infection of B lymphocytes in vitro requires at least 6 viral genes (EBNAs 1, 2, 3A, 3C, LP, in LMP1) for cell immortalization\(^12\). In in vivo infections this expression seems to be characteristic for infectious mononucleosis\(^13\). Three modes of EBV latency have been defined\(^14\). Specifically in EBV-positive Burkitt’s lymphomas latency program I is expressed, permitting only the synthesis of the Epstein–Barr virus nuclear antigen (EBNA)-1, transcripts clustered around one other open reading frame, BARF-0, and a small abundant non-translated RNA, EBER. EBNA-1 is required for EBV DNA replication and to permit the persistence of episomal viral DNA, the role of transcripts in the BARF-0 region is still not elucidated. Interestingly, recent data seem to point to a specific role of EBER transcripts in preventing apoptosis (Takada, pers. commun). In most other EBV containing malignant tumors (nasopharyngeal cancer, EBV-positive Hodgkin’s lymphomas and gastric cancers) latency program II is expressed, corresponding to the previous one, but expressing in addition to a varying degree the latency membrane proteins (LMP) 1 and 2. Latency program III finally is observed in EBV-induced lymphoblastoid proliferation and in EBV-positive B cell lymphomas arising under conditions of immunosuppression. In this case gene regulation occurs from a different promoter (Cp/Wp) and results in the expression of 6 different EBNAs proteins, three latent membrane proteins and EBER RNA. The EBNA-2 protein represents a specific transactivator of cellular and viral genes. It binds to the cellular protein RBP-Jc which acts downstream of the Notch-signaling pathway and transforms this protein from a repressor into an activator\(^15\,\,^16\).
The mechanism by which EBV proteins contribute to
malignant tumors is still poorly understood. This accounts
in particular for tumors expressing latency programs I or
II. EBV-positive lymphomas developing in immunosup-
pressed patients emerge as the result of a failing immune
system and the potent transactivating and growth-
stimulating activity of EBNA-2.

HHV-8 is the most recently identified human tumor
virus. Its genome contains a number of genes whose
products are related to cellular cyclins (cyclin D), to cyto-
kines (II-6) and to interferon-responsive factors (IRF-2).
Their accurate contribution to growth-stimulation and cell
transformation is presently under intensive study.

HTLV-I and also a related retrovirus, not yet linked
to human tumors, HTLV-II, are both able to immortalize human
T lymphocytes. This property seems to relate to a specific
viral gene tax that has been identified as a transform-
ing factor, possessing strong transactivating properties.

High risk human papillomavirus, particularly well stud-
ied HPV 16 and 18, code for three viral oncoproteins. One
of them, E5, is obviously not required to initiate and main-
tain the malignant phenotype of cervical carcinoma cells. It
seems to play, however, a role in early growth stimulation
of cells infected by these viruses. The two other oncoproteins,
E6 and E7, of high risk types are able to immortalize human
keratinocytes in contrast to low risk types. They contain
all the necessary information for cell immortalisation, although
infection of susceptible cells or transfection of
these genes per se is not sufficient for the induction of an
limited in vitro life span. The E7 protein interacts with
the retinoblastoma susceptibility gene product pRb (ref. 26)
and other pRb-related proteins. As a consequence, it inter-
rupts a complex between pRb and E2F, releasing the E2F
transcription factor which activates genes engaged in cell
cycle progression. Additional binding activities have been
described for E7 whose functional importance has not yet
been clearly established. The E6 protein binds p53 and abro-
gates its tumor suppressive and transcriptional activation
properties. It promotes ubiquination of p53 and its sub-
sequent proteolysis through interaction with the E6AP
ubiquitin–protein ligase. E6 and E7 are able to immor-
talise human keratinocytes independently, although both
genesis co-operate effectively in immortalisation events. As
observed for E7, E6 also targets other proteins: the focal
adhesion protein paxillin and the interferon regulatory
factor 3 (IRF-3), blocking the induction of interferon-
βmRNA after viral infection. These data indicate the multi-
functionality of viral oncoproteins, modifying a multitude
of cellular functions. Table 2 summarizes known human
tumor-viruses.

Protective mechanisms of the host against
potential tumor-viruses – the CIF-concept

The discussion on the role of EBV in Burkitt’s lymphoma
has been controversial for more than 30 years. Recently
some direct evidence has been reported pointing to an EB
viral contribution to the malignant phenotype of these cells.
Loss of EBV genomes was noted in the BL line Akata. EBV
negative clones lost the tumorigenic phenotype which
was reconstituted by re-introduction of EBV DNA.

Evidence was obtained recently for the host regulation of
persistent EBV infections by the genetic analysis of a
rare EBV-linked condition, the X chromosome-linked
lymphoproliferative syndrome (XLP). In XLP patients
the host is unable to cope with the B cell proliferation
which characterizes the initial stage of infectious mono-
nucleosis, a disease caused by acute EBV infection. XLP
patients succumb from an enormous lymphoproliferation.
The condition affects exclusively young males as has been
linked to deletions of the X chromosome. Recently mutations
have been identified in an inhibitor of a protein
(SLAM) that regulates T/B cell interactions. The gene
for the inhibitor protein, SLAM-associated protein or
SAP, was identified at the site of the X chromosome
deleted in XLP patients. A subsequent analysis demon-
strated its mutation specifically in this group of patients.
It is presently not understood, how this disturbance in T/B
cell relatively selectively affects the T cell control of EBV
infections.

Probably the best evidence for host defense mech a-
nisms against a family of tumor-viruses, even beyond a
mere immunological control, has been derived recently
from studies on human papillomavirus infections. The
papillomavirus family reveals an enormous complexity
with 85 fully analysed genotypes and more than 120 addi-
tional putative genotypes which are only partially charac-
terized up to now (de Villiers, pers. commun.). It
seems that the peculiar mode of papillomavirus propaga-
tion at cutaneous and mucosal surfaces only marginally
exposes these viruses to the immune system of the host.
This may release these infections from immunological
constraints acting on other systemic infections and may in
part account for the puzzling multitude of HPV geno-
types.

Table 2. Established human tumor-viruses and human viruses
causing tumors only under experimental conditions

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<thead>
<tr>
<th>Directly carcinogenic viruses</th>
<th>Indirectly carcinogenic viruses</th>
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<tr>
<td>Epstein-Barr virus</td>
<td>HPV</td>
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<tr>
<td>Human herpes-virus type 8</td>
<td>Hepatitis B (?)</td>
</tr>
<tr>
<td>Several anogenital papillomavirus types (e.g. HPV 16, HPV 18 and others)</td>
<td>Some cutaneous papillomavirus types (?)</td>
</tr>
<tr>
<td>HTLV-I</td>
<td>Human viruses carcinogenic in experimental animals</td>
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<tr>
<td>Hepatitis B (?)</td>
<td>Human polyomaviruses BK and JC</td>
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<td></td>
<td>Several types of human adenoviruses (e.g. adenovirus type 12, type 18 and others)</td>
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Previous studies showed that E6/E7 gene expression of high risk HPV types is necessary for the initiation and maintenance of the immortalized but also for the malignant phenotype of HPV DNA-carrying cells. These findings, combined with additional observations demonstrated that the expression of viral oncoproteins and transcription of viral oncogenes is necessary but not sufficient for the immortalized and malignant state of HPV-infected cells. Somatic cell hybrids between HPV-immortalized clones or between SV40-immortalised clones revealed the existence of complementation groups complementing each other to cellular senescence in spite of continuing E6/E7 mRNA or SV40 T-antigen synthesis. Therefore, a hypothesis was put forward postulating a cellular control of viral oncogene transcription or viral oncoprotein function, preventing in proliferating cells of the natural host their potential transforming and thus deleterious effect for the host. For high risk HPV infections a possible transcriptional control received experimental support from data showing suppression of HPV transcription upon inoculation of immortalized cells into immunocompromised animals and from a barely detectable level of E6/E7 transcripts in most low grade cervical intraepithelial lesions in contrast to high grade dysplasias. The initial suspicion of one cellular interfering factor (CIF) exhibiting an intracellular control beyond an immunological surveillance had to be modified into a CIF-concept.

Today there exists good evidence in support of the CIF-concept: at least two signaling cascades emerge regulating the transcription of high risk HPV oncogenes and interfering with the function of viral oncoprotein. A functional control can be deduced from somatic cell hybridization studies revealing continued HPV mRNA synthesis in senescent hybrids of initially immortalized clones. Transfection of human keratinocytes with the HPV 16 E6 oncogene only results in immortalized clones that generally contain mutated or methylation-silenced sequences of the p16 cyclin-dependent kinase inhibitor (Whitaker and zur Hausen, unpublished data). This suggests an important role of p16 in the control of E6-mediated cell immortalization. The regulatory steps engaged here are still not understood. For E7 as well as for E6 and E7 immortalized cells the obviously existing functional interference is even less understood. A high level of p21 and p27 may negatively interfere with a low level of E7 expression, at least dysregulated E7 expression in turn is able to inactivate these cyclin-dependent kinase inhibitors reciprocally.

The CIF-cascade interfering with the transcription of persisting high risk HPV is somewhat better understood. Exposure of HPV 16- or 18-immortalised cells to macrophages or treatment with tumor necrosis factor (TNF-α) leads to a selective suppression of HPV transcription. The effect is absent in HPV-containing malignant cervical cancer cells. Suppression in HPV transcription is accompanied by a remarkable shift in the composition of the AP-1 transcription factor as one of the important regulators of the HPV genome activity. In immortalized cells in tissue culture predominantly c-jun/c-fos heterodimers form this complex, whereas in most malignant lines c-jun/c-fos heterodimers prevail. TNF treatment results in the induction of a c-fos analogon, Fra-1, which now heterodimerizes with a phosphorylated form of c-jun. Malignant cells neither reveal Fra-1 induction nor an increase in c-jun/Fra-1 heterodimers. Although not directly proven, the available data suggest that the change in the AP-1 composition is responsible for the observed selective inhibition of HPV transcription in immortalized cells.

Presently a tentative CIF-cascade can be deducted from data available in the literature: there exists evidence that the protein phosphates 2A (PP2A) plays an important role in the regulation of HPV transcription. This is derived from data published by Smits et al. who demonstrated that human cells carrying a deletion in the short arm of chromosome 11 revealed a up-regulation of the regulatory component of PP2A, the PR55β protein. The resulting down-regulation of PP2A function that was also achieved by oncogenic acid treatment or by the introduction of SV40 small t-antigen led to increased transformability of these cells by high risk HPV DNA transfection and high levels of E6/E7 gene transcription. Preliminary data from our laboratory suggest that conditional up-regulation of E7 expression in turn activates the PR55β gene, pointing to a positive feedback mechanism (Hoffmann and zur Hausen, unpublished data). The activation of MKK6/p38 kinases by TNFα (ref. 56) may point to an important role of these kinases in the downstream events resulting in up-regulation of Fra-1 and c-jun.

The data reported here reveal a control of persisting natural tumor-virus infections that are not readily reached by the immune system by intra- and intercellular signaling cascades. In these instances cancer represents an accident resulting from modifications of host cell genes involved in the control of viral oncogenes and oncoproteins. In high risk HPV infections the viral oncoproteins, in addition to their gene regulatory functions appear to act as mutagens and may thus act as solitary carcinogens.

**Perspectives for cancer prevention and cancer therapy**

The discovery of infectious agents as causative factors for specific human cancers has important consequences for cancer prevention. This is presently apparent from vaccination studies performed in Taiwan and The Gambia. The Taiwan vaccination program of newborn children against hepatitis B infections, introduced in 1986, not only drastically reduced the percentage of persistently HBV-infected children, but also resulted in a first measurable decrease in liver cancer incidence. Presently clinical trials determine the efficacy of vaccines directed against high risk papillomavirus infections. Preceding
tests in animal papillomavirus infections, based on analogous vaccine preparations revealed a remarkable efficiency. The first tests in human clinical trials revealed the safety of the vaccine and the induction of high titers of neutralizing antibodies. Provided the vaccines against human high risk HPVs prove to be similarly effective as in animal experiments, one can estimate that a global application of HPV and HBV vaccines could theoretically reduce the cancer risk in women by approximately 15%.

Clinical trials are also presently conducted to test the potential of therapeutic vaccines. Their chances for effectiveness may more concern pre-malignant lesions rather than fully invasive tumors, although this remains presently difficult to predict.