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Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease

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The RNA and DNA tumor viruses have made fundamental contributions to two major areas of cancer research. Viruses were vital, first, to the discovery and analysis of cellular growth control pathways and the synthesis of current concepts of cancer biology and, second, to the recognition of the etiology of some human cancers. Transforming retroviruses carry oncogenes derived from cellular genes that are involved in mitogenic signalling and growth control. DNA tumor viruses encode oncogenes of viral origin that are essential for viral replication and cell transformation; viral oncoproteins complex with cellular proteins to stimulate cell cycle progression and led to the discovery of tumor suppressors. Viral systems support the concept that cancer development occurs by the accumulation of multiple cooperating events. Viruses are now accepted as bona fide etiologic factors of human cancer; these include hepatitis B virus, Epstein–Barr virus, human papillomaviruses, human T-cell leukemia virus type I and hepatitis C virus, plus several candidate human cancer viruses. It is estimated that 15% of all human tumors worldwide are caused by viruses. The infectious nature of viruses distinguishes them from all other cancer-causing factors; tumor viruses establish long-term persistent infections in humans, with cancer an accidental side effect of viral replication strategies. Viruses are usually not complete carcinogens, and the known human cancer viruses display different roles in transformation. Many years may pass between initial infection and tumor appearance and most infected individuals do not develop cancer, although immunocompromised individuals are at elevated risk of viral-associated cancers. Variable factors that influence viral carcinogenesis are reviewed, including possible synergy between viruses and environmental cofactors. The difficulties in establishing an etiologic role for a virus in human cancer are discussed, as well as the different approaches that proved viral links to cancer. Future directions for tumor virus studies are considered.

Abbreviations: AIDS, acquired immunodeficiency syndrome; ATL, adult T-cell leukemia; BKV, BK virus; BL, Burkitt's lymphoma; EBNA, Epstein–Barr virus-encoded nuclear antigen; EBV, Epstein–Barr virus; EV, epidermodysplasia verruciformis; HAM/TSP, HTLV-I-associated myelopathy/tropical spastic paraparesis; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HHV-8(KSHV), human herpesvirus type 8 (Kaposi's sarcoma-associated herpesvirus); HIV, human immunodeficiency virus; HPV, human papillomavirus; HTLV-I, human T-cell leukemia virus type I; JCV, JC virus; KS, Kaposi's sarcoma; MCV, molluscum contagiosum virus; MHC, major histocompatibility complex; MMTV, mouse mammary tumor virus; NPC, nasopharyngeal carcinoma; PCR, polymerase chain reaction; RDA, representational difference analysis; RSV, Rous sarcoma virus; SV40, simian virus 40; T-ag, large T-antigen; TRAF, tumor necrosis factor receptor-associated factors; VLP, virus-like particle.

Introduction

Viruses have been key instruments in the revolution in cancer biology that has occurred over the last 20 years. Without the contributions of viral carcinogenesis, it is difficult to conceive that the molecular basis of cancer would stand revealed so clearly today. Although viewed originally as unusual agents that caused cancer in animals but were of no particular relevance to humans, viruses have turned out to be the Rosetta stone for unlocking the mysteries of cell growth control. They have revealed the functional foundations of the genetic basis of cancer and have provided a conceptual framework applicable not only to cancer induced by viruses but to all neoplasia.

The tumor viruses have played two major roles in cancer research over the last 2 decades: first, as tools for the discovery and dissection of cell signalling and growth control pathways and, second, as newly appreciated causative agents of human neoplasia. The RNA tumor viruses have figured prominently in the first field of endeavor, whereas the DNA tumor viruses have been principals in both areas. This article will be an interpretation of major developments and conceptual changes that have been shaped by viral carcinogenesis over the past 20 years. Due to space constraints, it is not possible to present the fascinating details of specific virus-cancer systems, but recent publications may be consulted for in-depth descriptions of the characteristics of individual viruses, properties of different tumor virus systems and mechanisms of action of viral transforming genes (1–5). The biochemical and molecular details of cellular processes that may be affected by tumor viruses are covered in other articles in this issue (6).

Conceptual advances

Viral carcinogenesis provided the breakthroughs that crystallized current concepts of cancer development (Table I) and revealed mechanisms responsible for the orchestration of normal cell growth control. Both the RNA tumor viruses and the DNA tumor viruses played pivotal roles in the establishment of paradigms that extend far beyond virology to form the foundation of contemporary cancer biology.

Oncogenes

Transforming retroviruses carry cellular genes. All retroviruses share the unusual characteristic of reverse transcription in their life cycle. Soon after infection, the viral RNA genome is transcribed by a virion-associated enzyme, the reverse transcriptase, into a double-stranded DNA copy which is then integrated into the chromosomal DNA of the cell with the help of the viral integrase enzyme. There are many possible sites for proviral integration in the cellular genome. The integrated copy, termed the provirus, is similar to a cellular gene except that transcription is usually controlled by sequences

Table I. Concepts in cancer biology advanced by viral carcinogenesis

1.	Cellular origin of viral oncogenes—oncogenes carried by transforming retroviruses are derived from cellular genes
2.	Genetic basis of cancer—oncogenes involved in cancer development are aberrant versions of normal cell genes that function in growth control
3.	Multistep carcinogenesis—multiple genetic changes are required for tumor development
4.	Positive and negative regulatory genes as cancer genes—both proto-oncogenes and tumor suppressor genes are altered in cancer cells
5.	Unification of molecular basis of cancer—different classes of carcinogens affect the same regulatory network of cellular growth control pathways

in the viral long terminal repeat. Retroviral infection of a cell is permanent, as proviruses are almost never lost from the chromosome.

Rous sarcoma virus (RSV), the transmissible cause of a chicken sarcoma isolated by Rous in 1911 (7), together with basic virology studies in the 1960s provided the tools that led to the discovery of oncogenes (8). The development of a quantitative *in vitro* focus assay for RSV (9) permitted genetic studies that showed that replication and transformation represented separate viral gene functions and that the transforming gene (*src*) was dispensable for viral growth. Following the revolutionary discovery of reverse transcriptase (10,11), a probe specific for the *src* gene was prepared and, most unexpectedly, was found to be able to hybridize to normal cell DNA (12). The implications of this observation, that a tumor virus contained a gene related to a cellular gene, were enormous.

Numerous animal retroviruses with oncogenic capabilities had been isolated in the 1950s and 1960s, primarily from chickens and mice (8) (Figure 1). All the oncogenes carried by those transforming retroviruses were subsequently shown to be derived from the cell. The progenitor cellular genes, referred to as proto-oncogenes, have been identified as classes of genes involved in mitogenic signalling and growth control, including protein kinases, growth factor receptors, growth factors, G proteins, transcription factors and adapter proteins (Table II) (13). The observation that first linked an oncogene from a chicken sarcoma virus with a cellular transcription factor was quite unanticipated (14). More than 30 transduced oncogenes in transforming retroviruses have been identified (15).

The probable mechanism of oncogene transduction is shown in Figure 2. Integration of a provirus upstream of a proto-oncogene may produce chimeric virus–cell transcripts, and recombination during the next round of replication could lead to incorporation of the cellular gene into the viral genome (16). Typically, the inserted cellular sequences are copies of spliced transcripts, containing no introns. Viral genes usually are lost as a result of this capture process, yielding viruses that are defective for replication and dependent on replication-competent helper virus within the same cell to provide viral functions necessary for replication. Acutely-transforming retroviruses carrying oncogenes do not induce significant numbers of naturally occurring tumors. Not only is oncogene piracy a rare event, it is doubtful such viruses would survive long in nature because of their defective phenotype. The legacy of the known transforming retroviruses was assured by the laboratory studies of tumor virologists.

Retroviruses may be categorized as simple or complex, based on their genomic organization (Figure 1). Only the simple retroviruses have been found to transduce cellular genes; complex retroviruses [e.g., human T-cell leukemia virus type I (HTLV-I), bovine leukemia virus, human immunodeficiency virus (HIV)] have not. Perhaps the more complicated genetic organization of the complex viruses is less able to tolerate foreign inserts and retain fitness to be packaged and replicated, or perhaps available laboratory systems have simply failed to detect such rare mutants.

Viral oncogenes have usually been mutated in some way during their acquisition from the cell. Once incorporated into the viral genome, an oncogene is freed from normal cellular constraints and is expressed constitutively in transduced cells under the control of the viral long terminal repeat. In addition, there is the possibility that the transducing retrovirus will infect a cell type that does not normally express the proto-oncogene and lacks controls to regulate it. This combination of events, overexpression or inappropriate expression of a modified growth-related gene, leads to malignant transformation of the target cell. Retroviruses carrying transduced oncogenes can usually transform cells in culture and induce tumors after short latent periods in animals.

Non-transforming retroviruses activate cellular proto-oncogenes. Many retroviruses that do not possess viral oncogenes [e.g., avian leukosis virus, mouse mammary tumor virus (MMTV)] can induce tumors in animals (Figure 1). They do so by integrating a provirus near normal cellular proto-oncogenes and activating their expression, by a mechanism termed ‘proviral insertional mutagenesis’. The insertion of the provirus introduces strong promoter and enhancer sequences into the gene locus, and these changes modify gene expression (17).

Over 70 proto-oncogenes activated by proviral insertion of a non-transforming retrovirus have been identified (Table III). This number includes some genes first identified as viral oncogenes (15). These retroviruses that lack oncogenes are replication-competent, do not transform cells in culture and induce tumors with long latent periods *in vivo*. There is ongoing replication of these viruses in the host during the latent period before tumor development and, presumably, a chance event may place a provirus near a cellular oncogene. Altered expression of a growth regulatory gene by the acquired provirus may give that cell a selective growth advantage and ensure its survival while, over time, additional genetic changes accumulate. Tumors that arise after the long latent period are clonal, consistent with a series of rare events that cooperate to produce a transformed cell able to multiply and form a tumor. Clearly, the ability of a virus to replicate well and infect large numbers of cells in the target organ increases the chance that a gene involved in growth control will eventually get subverted.

The majority of tumors induced by the non-transforming retroviruses in chickens, mice and cats involve blood cells. The same oncogene is not activated by proviral insertion in all examples of a given type of tumor (15), illustrating that different biochemical changes at the cellular level can result in the same pathology. However, there are some examples in which a given oncogene is frequently altered [e.g., c-*myc* in avian leukosis virus-induced bursal lymphomas; N-*myc* in woodchuck hepatitis virus-induced liver tumors (18)]. MMTV is a retrovirus that induces solid tumors (carcinomas) and is

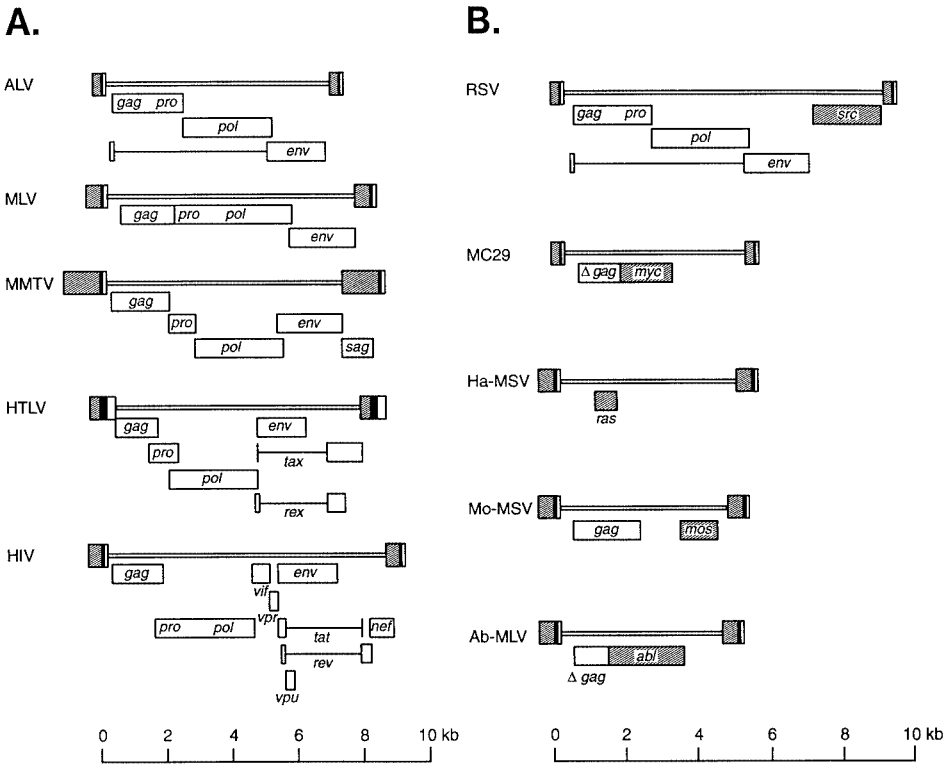

Fig. 1. Genetic organization of representative retroviruses. (**A**) Non-defective, replication-competent viruses. Examples of retroviruses with simple and complex genomes are shown. An open rectangle shows the open reading frame for the indicated gene. If the rectangles are offset vertically, their reading frames are different. Horizontal lines connecting two rectangles indicate that this segment is spliced out. Simple genomes: avian leukosis virus (ALV), murine leukemia virus (MLV) and MMTV. Complex genomes: HTLV and HIV. (**B**) Viruses carrying oncogenes. Several examples are shown, with the oncogene shaded; all are defective except RSV. MC29, avian myelocytomatosis virus (*myc* oncogene); Ha-MSV, Harvey murine sarcoma virus (*ras* oncogene); Mo-MSV, Moloney murine sarcoma virus (*mos* oncogene); Ab-MLV, Abelson murine leukemia virus (*abl* oncogene). The scale for genome sizes is shown at the bottom of each panel. Modified, with permission, from Vogt (167).

Table II. Retroviruses containing cellular oncogenes[a]

General class	Oncogene	Virus			Protein product
		Name	Abbreviation	Origin	
Non-receptor protein tyrosine kinase	*abl*	Abelson murine leukemia virus	Ab-MLV	Mouse	Tyrosine kinase
	fes	ST feline sarcoma virus	ST-FeSV	Cat	Tyrosine kinase
	fps	Fujinami sarcoma virus	FuSV	Chicken	Tyrosine kinase
	src	Rous sarcoma virus	RSV	Chicken	Tyrosine kinase
Receptor protein tyrosine kinase	*erbB*	Avian erythroblastosis virus	AEV-ES4	Chicken	Epidermal growth factor receptor
	fms	McDonough feline sarcoma virus	SM-FeSV	Cat	Colony-stimulating factor receptor
	kit	Hardy–Zuckerman-4 feline sarcoma virus	HZ4-FeSV	Cat	Stem cell factor receptor
Serine/threonine protein kinase	*mil*	Avian myelocytoma virus	MH2	Chicken	Serine/threonine kinase
	mos	Moloney murine sarcoma virus	Mo-MSV	Mouse	Serine/threonine kinase
	raf	Murine sarcoma virus 3611	MSV3611	Mouse	Serine/threonine kinase
Growth factor	*sis*	Simian sarcoma virus	SSV	Monkey	Platelet-derived growth factor
G protein	*H-ras*	Harvey murine sarcoma virus	Ha-MSV	Rat	GDP/GTP binding
	K-ras	Kirsten murine sarcoma virus	Ki-MSV	Rat	GDP/GTP binding
Transcription factor	*erbA*	Avian erythroblastosis virus	AEV-ES4	Chicken	Transcription factor (thyroid hormone receptor)
	ets	Avian myeloblastosis virus E26	AMV-E26	Chicken	Transcription factor
	fos	FBJ osteosarcoma virus	FBJ-MSV	Mouse	Transcription factor (AP1 component)
	jun	Avian sarcoma virus-17	ASV-17	Chicken	Transcription factor (AP1 component)
	myb	Avian myeloblastosis virus	AMV	Chicken	Transcription factor
	myc	MC29 myelocytoma virus	MC29	Chicken	Transcription factor
	rel	Reticuloendotheliosis virus	REV-T	Turkey	Transcription factor (NF-κB family)
Adapter protein	*crk*	CT10 avian sarcoma virus	CT-10	Chicken	

[a]Adapted from Rosenberg and Jolicoeur (15). This list is representative, not exhaustive.

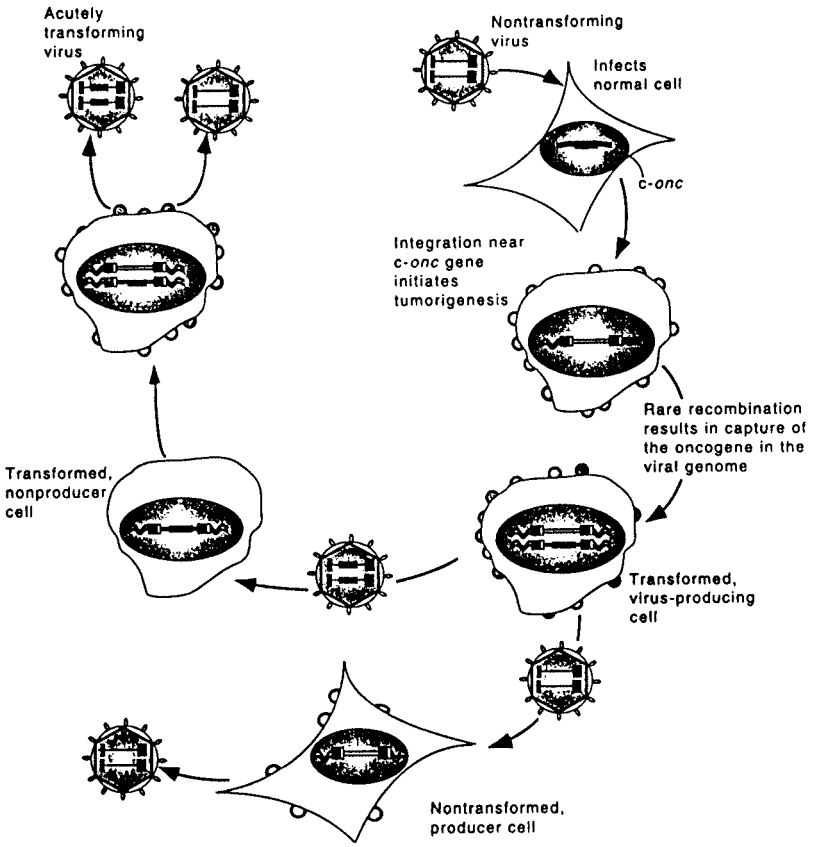

Fig. 2. Retroviral transformation and oncogene transduction. Clockwise from the top right: infection of a cell with a retrovirus that contains only viral genes can occasionally initiate tumor formation by insertion of the provirus next to a proto-oncogene. Recombination during subsequent infection can lead to the incorporation into the viral genome of the cell-derived oncogene. Cells infected with virus with only the oncogene-containing genome become transformed non-producer cells from which transforming virus can be rescued by superinfection with a replication-competent, non-transforming helper virus. Reproduced, with permission, from Vogt (8).

Table III. Cellular oncogenes activated by insertion of retroviruses lacking oncogenes[a]

General class	Oncogene	Virus			Protein product
		Name	Abbreviation	Origin	
Non-receptor protein tyrosine kinase	*Lck*	Moloney murine leukemia virus	Mo-MLV	Mouse	Tyrosine kinase
Receptor protein tyrosine kinase	*c-erbB*	Rous-associated virus 1	RAV-1	Chicken	Epidermal growth factor receptor
	c-fms	Friend murine leukemia virus	Fr-MLV	Mouse	Colony stimulating factor receptor
Serine/threonine protein kinase	*Pim1*	Moloney murine leukemia virus	Mo-MLV	Mouse	Serine/threonine kinase
Growth factor	*Fgf3/Int2*	Mouse mammary tumor virus	MMTV	Mouse	Fibroblast growth factor
	Wnt1/Int1	Mouse mammary tumor virus	MMTV	Mouse	Secreted glycoprotein
	Wnt3/Int4	Mouse mammary tumor virus	MMTV	Mouse	Secreted glycoprotein
G protein	*c-Ki-ras*	Friend murine leukemia virus	Fr-MLV	Mouse	GDP/GTP binding
Transcription factor	*Ets1*	Moloney murine leukemia virus	Mo-MLV	Rat	Transcription factor
	c-fos	Rous-associated virus 1	RAV-1	Chicken	Transcription factor (AP1 component)
	c-myb	Rous-associated virus 1	RAV-1	Chicken	Transcription factor
	c-myb	Moloney murine leukemia virus	Mo-MLV	Mouse	Transcription factor
	c-myc	Rous-associated virus 1	RAV-1	Chicken	Transcription factor
	c-myc	Moloney murine leukemia virus	Mo-MLV	Mouse	Transcription factor
Cyclin	*Fis1/Cyclin D1*	Friend murine leukemia virus	Fr-MLV	Mouse	G_1 cyclin
	Vin1/Cyclin D2	Moloney murine leukemia virus	Mo-MLV	Mouse	G_1 cyclin

[a]Adapted from Rosenberg and Jolicoeur (15). This list is representative, not exhaustive.

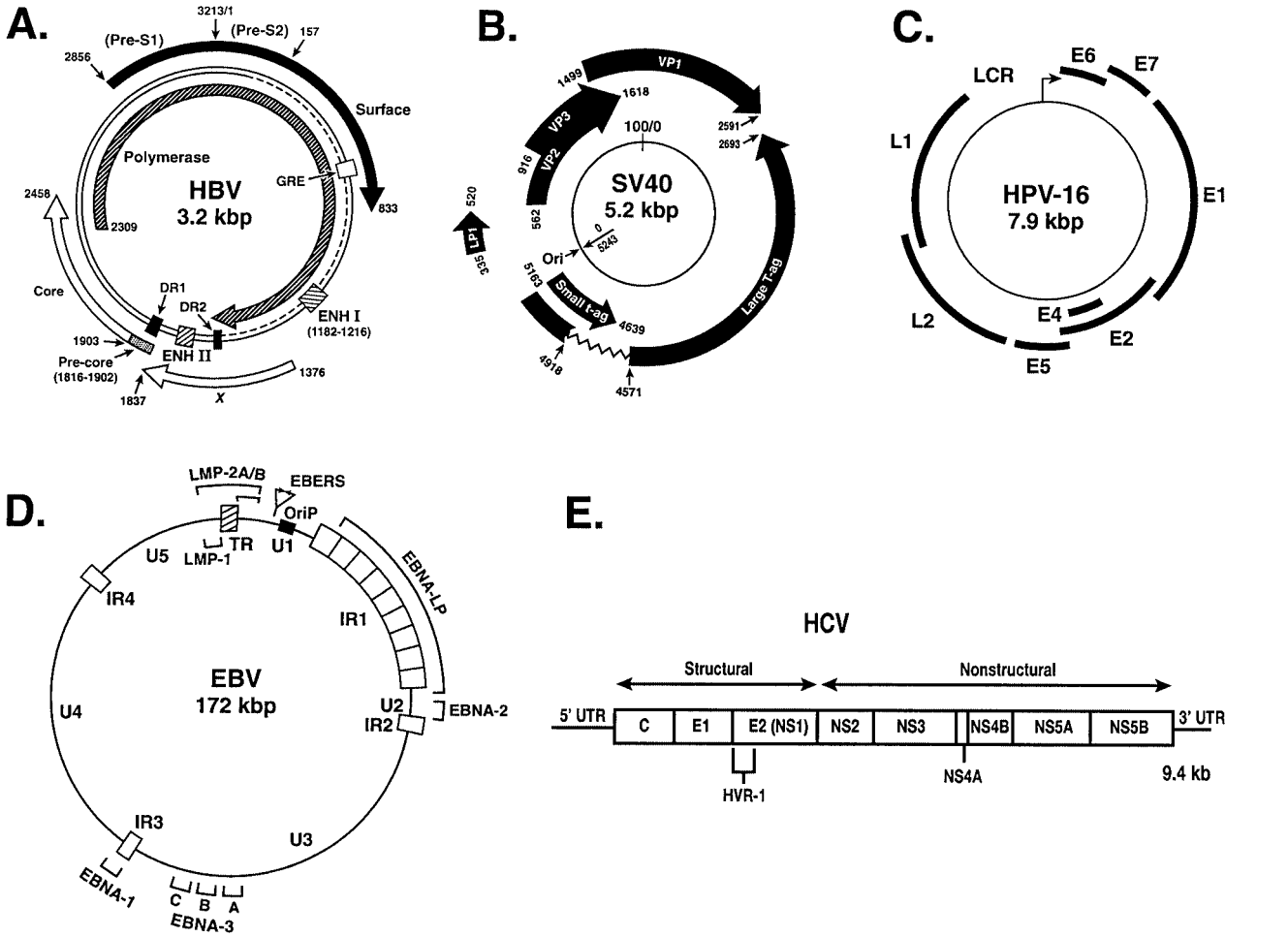

Fig. 3. Genome maps of human tumor viruses. (**A**) HBV. The partially double-stranded HBV genome, consisting of a long (–) and a short (+) strand of DNA, is represented by the solid and partially broken lines, respectively. Four arrows depict the functional ORFs of the HBV genome, which are designated surface, polymerase, core and X. Nucleotide numbers for the boundaries of each ORF are given. The map positions for the two viral enhancers (ENH I and ENH II), the viral direct repeats (DR1 and DR2) and the glucocorticoid-response element (GRE) are shown. [Modified from Butel *et al*. (121).] (**B**) SV40. The circular SV40 DNA genome is represented with nucleotide numbers based on reference strain SV40-776 beginning and ending at the origin (*Ori*) of viral DNA replication. The ORFs that encode viral proteins are indicated. Arrowheads point in the direction of transcription; the beginning and end of each ORF are indicated by nucleotide numbers. T-ag, the essential replication protein and viral oncoprotein, is coded by two non-contiguous segments on the genome. t-ag, small t-antigen. (Reproduced from Brooks,G.F., Butel,J.S. and Morse,S.A. (1998) *Med. Microbiol.* 21st edn, with permission from Appleton & Lange.) (**C**) HPV. The circular episomal DNA genome is shown for HPV-16. The early (E1–E7) and late (L1–L2) ORFs are shown as heavy black lines; the different reading frames are offset vertically. The long control region (LCR) contains the origin of DNA replication and the constitutive transcriptional enhancer. Redrawn with permission from Alani and Münger (168). (**D**) EBV. The circular DNA episome is shown. The largely unique sequence domains (U1–U5), internal repeats (IR1–4), the terminal repeat (TR), and the episomal replicon (oriP) are indicated. Latency-associated proteins found in infected B lymphocytes are shown: EBV nuclear proteins (EBNA1–3, LP) and latent membrane proteins (LMP1/2). EBERs are small non-polyadenylated RNAs. (**E**) HCV. The single ORF is expressed as a polyprotein that gets processed; the positions of structural and non-structural domains are shown. HVR-1 represents the highly variable region of an envelope glycoprotein. Redrawn with permission from Chung and Liang (81).

the cause of breast cancer in mice. Tumor induction in some mouse strains is associated with proviral insertional activation of a growth-factor-related gene, such as the gene for fibroblast growth factor (*Int2*) or *Wnt-1* (19). However, for many mammary tumors, the genes activated by MMTV insertion are unknown.

DNA tumor viruses target tumor suppressor proteins

The oncogenes of the small DNA tumor viruses (polyomaviruses, papillomaviruses, adenoviruses) were found to be of viral, not cellular, origin and to be essential for both viral replication and cell transformation (20–22). These properties are in striking contrast to those of the cellular-derived oncogenes carried by the transforming retroviruses. Studies of these DNA viruses led to the discovery of cellular tumor suppressor genes, a second group of genes (other than oncogenes) critically important in cancer development.

Due to their limited genetic content, the small DNA tumor viruses are dependent on the host cell machinery to replicate the viral DNA (Figure 3). Virus-encoded non-structural proteins stimulate resting cells to enter S phase to provide the enzymes and environment conducive for viral DNA replication. Simian virus 40 (SV40) large T-antigen (T-ag) represents such a highly multifunctional protein (Figure 4), being required both for initiation of viral DNA synthesis and for stimulation of cell entry into S phase (23). Because of this ability to usurp cell cycle control, it is the major transforming protein of SV40 (24–26). The oncoproteins of polyomaviruses, papillomaviruses and adenoviruses and cellular proteins targeted for functional interaction are shown in Table IV. Because of their marked multifunctionality, these viral oncoproteins cannot be compared directly to cellular oncogene products.

The binding of viral oncoproteins to cellular tumor sup-

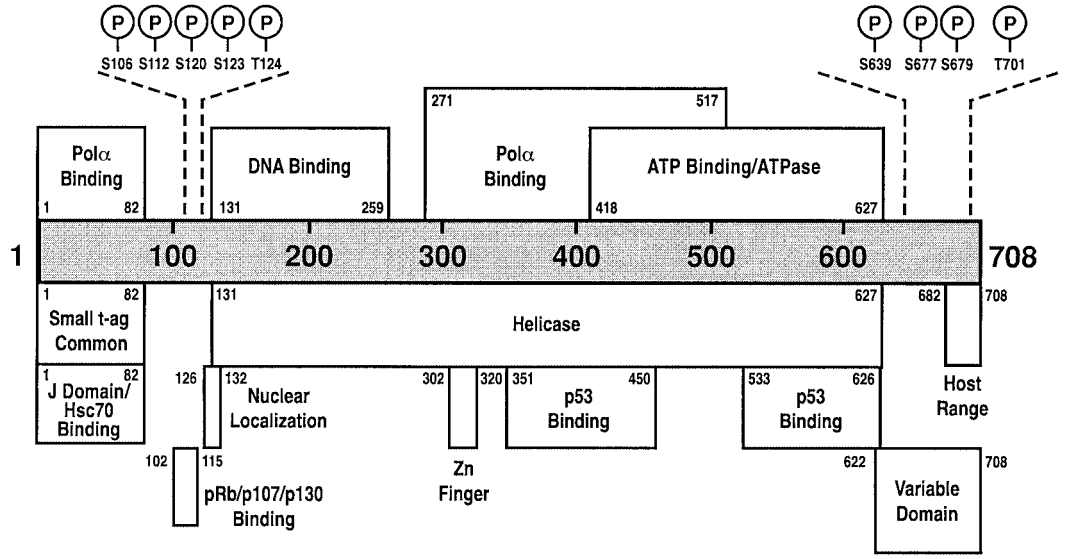

Fig. 4. Functional domains of SV40 large T-ag. The numbers given are the amino acid residues using the numbering system for SV40-776. Regions are indicated as follows: small t-ag common, region of large T-ag encoded in the first exon; the amino acid sequence in this region is common to both large T-ag and small t-ag. Polα binding, regions required for binding to polymerase α-primase; J domain/Hsc70 binding, region required for binding the heat shock protein hsc70; pRb/p107/p130 binding, region required for binding of the Rb tumor suppressor protein, and the Rb-related proteins p107 and p130; nuclear localization, contains the nuclear localization signal; DNA binding, minimal region required for binding to SV40 *Ori* DNA; helicase, region required for full helicase activity; Zn finger, region which binds zinc ions; p53 binding, regions required for binding the p53 tumor suppressor protein; ATP binding/ATPase, region containing the ATP binding site and ATPase catalytic activity; host range, region defined as containing the host range and adenovirus helper functions; variable domain, region containing amino acid differences among viral strains. The circles containing a P indicate sites of phosphorylation found on large T-ag expressed in mammalian cells. S indicates a serine and T indicates a threonine residue. Reproduced from Stewart *et al.* (169), with permission from *Virology.*

Table IV. DNA virus oncoproteins and cellular protein interactions

Virus	Viral oncoproteins	Cellular targets[a]
SV40	Large T-antigen	p53, pRb
	Small t-antigen	PP2A
HPV	E6	p53 via E6AP, DLG, MAGI-1, MUPP1
	E7	pRb
Adeno	E1A	pRb
	E1B-55K	p53
Adeno 9	E4ORF1	DLG, MAGI-1, MUPP1
BPV	E5	PDGFβ receptor
EBV	LMP1	TRAFs
HBV	HBx (?)	p53, DDB1
Polyoma	Large T-antigen	pRb
	Middle T-antigen	c-Src, PI3-K, PLC-γ, Shc
	Small t-antigen	PP2A

Adeno, adenovirus; BPV, bovine papillomavirus; PDGF, platelet-derived growth factor; PI3-K, phosphatidyl inositol-3 kinase; Polyoma, polyomavirus; PLC-γ, phospholipase C-γ; PP2A, protein phosphatase 2A.
[a]Additional cellular proteins are reported to interact with some of the viral oncoproteins. This list is representative, not exhaustive.

pressor proteins p53 and pRb is fundamental to the effects of the small DNA tumor viruses on host cells (20–22,27,28). As several different DNA tumor viruses encode unique oncoproteins that target pRb and p53, this emphasizes the central power these two proteins exert over cell growth control and the viral imperative to circumvent that control. The p53 protein was discovered as a cellular protein complexed with SV40 T-ag in SV40-transformed cells (29,30). A decade later, it was shown that normal p53 is not an oncogene (a gain-of-function activity that is acquired by some mutant forms of p53), but is in fact a tumor suppressor gene that inhibits cell growth (31). A second tumor suppressor protein, the retinoblastoma gene product, pRb, was identified as one of several host cell proteins complexed with the E1A oncoprotein in adenovirus-transformed cells (32). SV40 T-ag also forms complexes with pRb, and by binding to and abolishing the normal functions of these two cellular inhibitory proteins (p53, pRb) disrupts cell growth control mechanisms (20,24,25).

Briefly, the following scenario (oversimplified) is envisioned, using SV40-infected cells as an example. pRb normally binds transcription factor E2F in early G_1 in the cell cycle; when pRb is phosphorylated by cyclin-dependent kinases, E2F is released and functions to activate expression of growth stimulatory genes required for the cell to initiate DNA synthesis. T-ag causes unscheduled dissociation of pRb-E2F complexes, releasing active E2F. Wild-type p53 is believed to guard the integrity of the cellular genome (33) by inhibiting cell cycle progression or inducing apoptosis in response to aberrant proliferation signals, DNA damage or cellular stress. It acts as a key regulator of a complex circuitry involving MDM2, ARF and other cellular proteins (34–36). T-ag binding functionally inactivates p53, allowing cells inappropriately stimulated by the freed E2F to escape the p53 checkpoint, enter S phase and survive to replicate the viral DNA.

The DNA tumor viruses display different means of accomplishing the same end (i.e., abrogation of p53 checks on cell cycle progression). The unrelated viral oncoproteins bind to p53 through distinct sequences. When complexed to p53, SV40 T-ag inhibits its DNA binding activity, human papillomavirus (HPV) E6 targets it for ubiquitin-mediated degradation and adenovirus E1B-55K interferes with its transactivation function. These same tumor viruses also inactivate pRb by complex formation with viral oncoproteins. SV40 T-ag, HPV E7 and adenovirus E1A contain a short region of homology that includes a common amino acid motif, L–X–C–X–E, that is important for binding to pRb. The viral proteins display exquisite selectivity by homing in on the hypophosphorylated

form of pRb, the form that binds E2F, and discriminating against the hyperphosphorylated, presumably non-functional, forms. These same three viral proteins also bind other members of the pRb family, p107 and p130, the functional effects of which are less well understood. SV40 T-ag is the only viral oncoprotein able to interact with both p53 and pRb family members.

Other DNA viruses encode transforming proteins that target different cellular proteins. Human adenovirus 9 induces estrogen-dependent mammary tumors in rats, with the E4 ORF1 protein being the major oncoprotein in that system. That viral protein binds several cellular PDZ-domain proteins, including DLG, MAGI-1 and MUPP1 (37; R.T.Javier, unpublished data). PDZ domains are protein–protein interaction modules found in certain cell signalling proteins that localize to specialized membrane sites; the proteins are presumed to function as adapter proteins in signal transduction. The fact that E6 proteins from high-risk HPV types bind three of the same PDZ-domain proteins (37; R.T.Javier, unpublished data) suggests that those interactions may contribute to transformation via a previously unrecognized pathway. It remains to be established whether these PDZ-domain proteins have tumor suppressor properties.

The oncoprotein of mouse polyoma virus is the middle T-ag, a membrane-associated protein. It interacts with the c-Src protein (and other members of the Src kinase family), resulting in activation of the Src tyrosine kinase. Phosphorylation of tyrosine residues on middle T-ag permits binding to phosphatidyl inositol-3 kinase, phospholipase C-γ and Shc, which leads to activation of downstream signal transduction pathways and stimulation of cellular proliferation. Although SV40 and polyoma virus are similar viruses in many respects, they commandeer different regulatory pathways to prepare the cell for viral replication or to undergo transformation. Several other viral oncoproteins mediate transformation through mechanisms that do not involve tumor suppressor proteins, and these are discussed in the following section.

Unification of molecular basis of cancer

Twenty years ago, there was no unifying theory for the origin of cancer. Scientists working with tumor viruses, chemical carcinogens, or other carcinogenic substances were living parallel investigative lives, with each group assuming their system was unique. That all changed in 1982 when it was discovered that cellular transforming genes from human bladder and lung tumor cell lines, detected by laborious DNA transfection methodologies, were homologous to the *ras* genes already identified as oncogenes carried by transforming murine sarcoma viruses (38,39). Sequence analysis showed that a point mutation distinguished the bladder carcinoma oncogene from the normal cellular gene and that the mutation affected the identical codon as an activating mutation in the retrovirus v-*ras* oncogene. In the rat mammary carcinoma system involving the induction of mammary tumors by a chemical carcinogen, activated H-*ras* genes were detected at high frequency (40). Suddenly, it was apparent that the same cellular proto-oncogenes could be affected by viruses, by chemical carcinogens, or by non-viral somatic mutations; there were not specific groups of proto-oncogenes selectively targeted by different types of carcinogenic agents. Within a short period of time, several oncogenes previously identified in retroviruses were found to be mutated in human tumors. For example, chromosomal translocations in human acute leukemias most frequently affect genes encoding transcription factors, including the *myc* gene (41).

The recognition of tumor suppressor genes completed the unification of basic tumor virology and human oncology. The tumor suppressor proteins, identified as targets for inactivation by DNA tumor viruses, were found to be mutated or lost in many human tumors. Genetic alterations in p53 are now recognized as the most common mutations in human cancers, occurring in over half of all tumors (42,43). Again, it was apparent that different types of cancer-causing factors could affect the same cellular tumor suppressor genes or gene products. The wealth of literature from both the RNA tumor viruses and the DNA tumor viruses was made relevant to human cancer.

Oncogenes and tumor suppressor genes are a major focus of human cancer studies today, and additions to both classes of cancer genes that have no cognates among the tumor viruses have been identified. However, the precedent of alterations in normal cellular growth regulatory genes as the molecular basis of cancer was established using virus systems.

Multistep carcinogenesis

It is now accepted that carcinogenesis occurs in a step-wise fashion and that a series of discrete complementary events must occur to convert a normal cell into a cancer cell (44,45). Evidence for this biologic paradigm can be drawn from many sources, including from virus systems.

Cooperativity of transforming genes in vivo. A classic example of multistep carcinogenesis involved the cottontail rabbit papillomavirus. In experiments dating back 60 years, it was shown that when virus-induced papillomas were treated with coal tar, a large fraction of papillomas converted to carcinomas (46).

Some transforming retroviruses have pirated not one, but two cellular oncogenes into their genome, and these unusual transducers are more rapidly tumorigenic than their single-oncogene-containing cousins (15). This is illustrated by a particular isolate of avian erythroblastosis virus which possesses both the v-*erbA* and v-*erbB* oncogenes and is a more potent tumor-inducer *in vivo* than another isolate that carries only v-*erbB*. These observations suggest that there is functional cooperativity between oncogenes in their effects on cell phenotype *in vivo*. Even with the indirect-acting retroviruses, more than one oncogene has been found to be activated in some individual tumors. For example, in selected MMTV-induced mammary tumors, both *Wnt-1* and *Int2* genes have been observed to be activated by proviral insertions (15).

Cooperativity of transforming genes in vitro. Early evidence of cooperating oncogene activities was provided by *in vitro* transformation experiments (13,45). The first demonstration involved polyoma virus oncoproteins. Both large T-ag and middle T-ag were required for transformation of primary rat embryo fibroblasts, whereas middle T-ag alone was sufficient to transform established rat cell lines. The growth properties of transformants expressing only middle T-ag were serum dependent (47). Certain combinations of oncogenes (e.g., *myc* + *ras*, E1A + E1B, E1A + *ras*) were transforming in primary cultures of rodent cells, whereas the solitary oncogenes were not (48,49). When established cell lines were tested as substrates, however, some single oncogenes were overtly transforming (e.g., *ras*), indicating that permanent cell lines are more predisposed to transformation by certain oncogenes than are primary, more normal, cells.

Cooperativity of transforming events in transgenic mice. Transgenic mice have contributed greatly to studies of early steps in tumor development in whole animals. Many viral and cellular oncogenes when expressed in a target tissue induce preneoplastic lesions typified by widespread cellular hyperplasia; a limited number of discrete foci of neoplastic cells then arise out of this hyperplastic background (50,51). In transgenic mice with exogenous *ras* and *myc* expression directed to the mammary gland, doubly transgenic female animals reproducibly developed breast tumors much more rapidly than animals in either single-transgene parent line (50,52,53). These observations indicate that oncogene expression by itself is usually not adequate to induce frank tumor formation; the oncogene begins the process, but additional cooperating events are needed to complete cellular transformation.

The concept of cooperativity between transforming oncogenes has been extended to encompass the loss of tumor suppressor genes as cooperating events in oncogenesis. In this regard, studies with p53-deficient mice have firmly established the role of p53 in tumor suppression (54,55). There are now numerous examples of transgenic mice carrying activated oncogenes being crossed with p53-null animals and the p53-deficient, oncogene-positive progeny displaying accelerated tumor formation.

Cell fusion evidence of cooperating events in transformation. It is clear from somatic cell hybridization studies that additional genetic aberrations beyond the expression of viral transforming gene functions are required for immortalization of cells by SV40 or HPV (56–58). It has been shown using cell fusion techniques that the immortalized phenotype of virus-transformed human cells is a recessive trait, despite the continued expression of viral oncoproteins. Hybrids formed by fusion of virus-immortalized cells with either normal cells or immortal cells from a different complementation group exhibited limited division potential and senesced. These properties indicate the loss of function of a few specific cellular genes in the immortal human cells, the absence of which removes constraints on cell proliferation. Those genes have not been identified, but a growth suppressor gene on chromosome 6 has been found using SV40-immortalized human diploid cells (58) and a senescence gene on chromosome 4 has been detected (59). In addition, chromosomal changes have been reported in HPV-immortalized keratinocytes, with loss of heterozygosity preferentially involving sites on chromosomes 3 and 18 (60).

Anti-apoptosis as a step in carcinogenesis. Apoptosis, or programmed cell death, is a highly orchestrated process whereby cells commit suicide in response to a variety of stimuli; it is an important cellular defense against viral infection and cancer development. Cellular endonucleases induced as part of the apoptotic response to damage inflicted by virus infection could degrade replicating viral DNA and block virus replication. Therefore, some viruses are known to encode proteins which suppress or delay apoptosis long enough to allow for production of progeny virus (61). Such 'death-prevention' viral genes could contribute to cancer development if they allowed cells that were destined to self-destruct to continue to proliferate. Several tumor-virus-encoded proteins reportedly exhibit apoptosis-inhibiting activity. The most well characterized of these is the adenovirus E1B-19K protein which appears to be functionally similar (although bearing limited sequence similarity) to the Bcl-2 family of cellular proteins, which are known to block p53-dependent apoptosis (62,63). As p53 is believed to induce apoptosis as part of its growth-suppression activity (64), the viral oncoproteins that bind and inactivate p53 (e.g., HPV E6, SV40 T-ag) increase the chance of cell survival. It can be generalized that one of the spontaneous cellular alterations necessary to permit complete transformation in other virus or oncogene systems probably prevents the normal damage-induced apoptotic response of the cell.

Viruses as agents of human cancer

The last 20 years have witnessed the general recognition that viruses are involved in the genesis of human cancer, coupled with a growing catalog of human tumor types that have a viral etiology.

Human tumor viruses—history and classification
Tumor viruses were first detected at the turn of the twentieth century, with the cell-free transmission of human warts by Ciuffo in 1907 (65), of chicken leukemia by Ellermann and Bang in 1908 (66) and of a chicken sarcoma by Rous in 1911 (7). These ground-breaking results were met with scepticism and judged to be irrelevant, because leukemia was not then considered to be a form of cancer, the chicken was viewed as too unrelated to humans to be meaningful and cancer in humans was not contagious, so transmissibility in chickens was not seen as applicable to human disease (8). Murine leukemia viruses were demonstrated to be transmissible to newborn animals by Gross in 1950 (67). The next 2 decades were the glory days of isolation of animal viruses, including many retroviruses having tumorigenic properties in animals. However, attempts to isolate similar viruses from humans were disappointingly negative, raising doubts that human cancer viruses existed. During this time, the human herpesvirus Epstein–Barr virus (EBV) was observed by electron microscopy in cultured cells from Burkitt's lymphoma (BL) in 1964 (68) and the hepadnavirus hepatitis B virus (HBV) virion was visualized in human sera positive for Australia antigen (now designated hepatitis B surface antigen) in 1970 (69). It would be many years before these two viruses were accepted as causative agents of human neoplasia. Significant detections of possible human tumor viruses during the last 20 years have included recovery of HTLV-I from cell lines from patients with T-cell lymphoma/leukemia in 1980 and 1982 (70,71), isolation of HIV from individuals with acquired immunodeficiency syndrome (AIDS) in 1983 and 1984 (72–74), cloning of hepatitis C virus (HCV) from infectious sera of patients with post-transfusion hepatitis in 1989 (75), and identification of DNA sequences of human herpesvirus type 8 (Kaposi's sarcoma-associated herpesvirus) [HHV-8(KSHV)] in an AIDS-associated Kaposi's sarcoma (KS) skin lesion in 1994 (76).

As the twentieth century draws to a close, viruses are now recognized as bona fide members of the group of agents known to be cancer-causing in humans. Specific viruses that are accepted as playing a causal role in certain human cancers (77), as well as several candidate human tumor viruses, are listed in Table V. These viruses will be used to illustrate principles of viral carcinogenesis that have emerged from a combination of laboratory and epidemiologic studies. Numerous viral isolates from primates and lower animals produce cancer in animal model systems, and these have been utilized to define mechanisms of viral carcinogenesis that form the

Table V. Accepted and candidate human tumor viruses and selected animal model cancer viruses

Virus family	Causal role in human cancer		Animal models
	Accepted	Potential	
Hepadnaviridae	HBV		WHV, GSHV
Herpesviridae			
Gammaherpesviruses	EBV	HHV-8(KSHV)	HVS
Papovaviridae			
Papillomaviruses	HPV (high-risk types)	HPV (other types)	CRPV, BPV
Polyomaviruses		SV40, BKV, JCV	SV40, BKV, JCV, Py
Adenoviridae			Multiple serotypes
Poxviridae			
Molluscipoxvirus		MCV	
Leporipoxvirus			RFV
Retroviridae			
Simple			ALV/ASV, MLV/MSV, FeLV/FeSV, MMTV
Complex	HTLV-I	HIV	BLV
Flaviviridae	HCV		

ALV/ASV, avian leukosis-sarcoma virus group; BLV, bovine leukemia virus; BPV, bovine papillomavirus; CRPV, cottontail rabbit papillomavirus; FeLV/FeSV, feline leukemia-sarcoma virus group; GSHV, ground squirrel hepatitis virus; HVS, herpesvirus saimiri; MDV, Marek's disease virus; MLV/MSV, murine leukemia-sarcoma virus group; Py, murine polyoma virus; RFV, rabbit fibroma virus; WHV, woodchuck hepatitis virus.

basis of much of our understanding today. Selected examples are included in Table V.

Taxonomy. From a taxonomic viewpoint, tumor viruses are distributed throughout two RNA virus families and all the DNA virus families (with the exception of the Parvoviridae). Parvoviruses may be excluded from the transforming group because, due to their simplicity, they lack functions to stimulate resting cells to enter the cell cycle. The most potent DNA-containing human cancer viruses are found in the hepadnavirus, herpesvirus and papovavirus families (Figure 3). As will be described, these three classes of viruses function in different ways to mediate cell transformation. Although human adenoviruses have been extremely useful laboratory models for studying molecular details of eukaryotic cell processes in normal and transformed cells, for reasons that are not clear there are no proven or candidate etiologic agents of human tumors from that group. Some poxviruses induce hyperplasias or benign tumors that usually regress spontaneously, such as human molluscum contagiosum virus (MCV), but members of that family are currently not considered to be important cancer viruses.

All the known RNA-containing tumor viruses are classified as retroviruses, with the exception of HCV which resembles a flavivirus (Figures 1 and 3). The many animal isolates of retroviruses were central to investigations that revealed the genetic basis of cancer (described above). Ironically, in contrast to their widespread representation as cancer-causing agents in animals, the only currently accepted human tumor virus from the retrovirus family is HTLV-I (except for HIV that predisposes to cancer indirectly by damaging the host immune system). There are no known human tumor viruses comparable with the acutely-transforming oncogenic retroviruses that transduce cellular oncogenes and are replication defective.

Viruses and human tumors. Viruses are associated with a variety of types of human malignancies. Both HBV and HCV cause hepatocellular carcinoma (HCC) (78–83); EBV is etiologically linked to BL, nasopharyngeal carcinoma (NPC), post-transplant lymphomas, Hodgkin's disease, and possibly other tumors (84–86); the HPVs are the cause of cervical cancer, skin cancers in patients with epidermodysplasia verruciformis (EV), and possibly head and neck cancers and other anogenital cancers (46,60,87,88); HTLV-I induces adult T-cell leukemia (ATL) (89,90); HHV-8(KSHV) may be causally related to KS, primary effusion lymphoma and multicentric Castleman's disease, a lympho-proliferative disorder (91,92); and SV40 has been associated with brain tumors, osteosarcomas and mesotheliomas (24,93). It is estimated that ~15% of all human tumors have a viral etiology (77,94). This number reflects predominantly two malignancies that contribute significantly to the worldwide cancer burden, cervical cancer in women (caused by HPV) and liver cancer (caused by HBV and HCV). The percentage of virus-related cancers is ~3-fold higher in developing countries than in developed countries (77), reflecting the higher prevalence of infection by the causative viruses and possible exposure to cooperating cofactors. When other human cancers are proven to be caused by viruses, as seems likely, this percentage of infection-related tumors will undoubtedly increase.

Some viruses are associated with a single tumor type (e.g., HBV), whereas others are linked to multiple tumor types (e.g., EBV); these differences presumably reflect the tissue tropism(s) of a given virus. A virus associated with human tumors may also produce non-neoplastic disease in some hosts. HTLV-I is the cause of HTLV-I-associated myelopathy or tropical spastic paraparesis (HAM/TSP) (90), a neurological disease that develops even more rarely in infected persons than ATL; EBV causes infectious mononucleosis in some young adults undergoing primary infection; HPVs cause a variety of benign hyperplasias; and both HBV and HCV cause hepatitis. The frequency of disease development varies widely, reflecting the basic characteristics of the particular virus and features of the virus–host relationship, including the age at primary infection.

Tenets of viral carcinogenesis

General tenets of viral carcinogenesis can be articulated, based on data accumulated over the last 20 years (Table VI). The majority of these principles were established in animal model

Table VI. Tenets of viral carcinogenesis

1.	Viruses can cause cancer in animals and humans
2.	Tumor viruses frequently establish persistent infections in natural hosts
3.	Host factors are important determinants of virus-induced tumorigenesis
4.	Viruses are seldom complete carcinogens
5.	Virus infections are more common than virus-related tumor formation
6.	Long latent periods usually elapse between initial virus infection and tumor appearance
7.	Viral strains may differ in oncogenic potential
8.	Viruses may be either direct- or indirect-acting carcinogenic agents
9.	Oncogenic viruses modulate growth control pathways in cells
10.	Animal models may reveal mechanisms of viral carcinogenesis
11.	Viral markers are usually present in tumor cells
12.	One virus may be associated with more than one type of tumor

systems, but they have generally been reaffirmed as viral involvements in human malignancies have been scrutinized and substantiated. The first tenet, that viruses can cause cancer in animals and humans, is a summation of decades of studies involving viruses and their possible role in malignancies. Given the tortuous history of viral carcinogenesis, this statement of fact is a tribute to the pioneer investigators who persevered in the face of scepticism and criticism. Etiologic associations of viruses with human cancers gained general acceptance only in the past 2 decades.

The virologic, epidemiologic and pathogenic properties of several proven and potential human cancer viruses are summarized in Table VII. The obvious diversity among the recognized human tumor viruses illustrates that the stated tenets of viral carcinogenesis are applicable across viral taxonomic boundaries. However, because of the heterogeneity among the viruses with respect to basic characteristics and pathogenesis of infections, not all the general themes apply to all virus-cancer systems.

Tumor viruses are infectious agents

The infectious nature of viruses distinguishes them from all other cancer-causing agents (such as chemicals, radiation and hormones). Consequently, the pathogenesis of a viral infection and the response of the host are integral to understanding how cancer might arise from that background. The dynamics of an infectious disease imposes an element of variability that makes establishing etiologic associations with cancer more difficult. The recognized tumor viruses tend to establish long-term persistent infections in humans, as compared with the self-limited infections typical of most common viral diseases. The extent of virus replication and resulting virus load in an infected person is determined by a combination of factors, including the properties of the infecting virus, the type of cell infected and the state of the host's immune system (95). Due to differences in individual genetic susceptibilities and host immune responses, levels of virus replication and tissue tropisms may vary among persons. A successful long-term relationship presents the possibility of virus genetic variation over time within an individual host.

Host response and viral evasion tactics. The response of the host exerts constant pressure on a chronic virus infection and, in defense, viruses may contain genes that have the potential to modulate such host responses (96). The herpesviruses and poxviruses appear to be treasure troves of such viral defense mechanisms. The HHV-8(KSHV) genome possesses a number of cellular regulatory gene homologues, including genes related to chemokines, cellular proliferation factors, intercellular signalling components and inhibitors of apoptosis (91,92,97). About one-third of the large genome of the human poxvirus, MCV, is devoted to genes that would presumably affect its interaction with the host (98). MCV encodes a novel intracellular defense molecule, a protein highly related to human glutathione peroxidase, a scavenger of reactive and toxic oxygen metabolites (99). It is possible this viral enzyme could protect against the toxic effects of diffused peroxide from phagocytic leukocytes and prevent induction of apoptosis of infected cells.

Viruses that establish persistent infections must avoid detection and recognition by the immune system that would eliminate the infection. Different viral evasion strategies have been identified (95,100,101), including: (i) restricted expression of viral genes and proteins that makes the infected cells nearly invisible to the host (e.g., EBV in B cells); (ii) infection of sites that seem relatively inaccessible to immune responses [JC virus (JCV) and herpes simplex virus in the central nervous system, HPV in the epidermis and polyomaviruses and cytomegalovirus in the kidney]; (iii) variation in viral antigens that allows escape from antibody and T-cell recognition (e.g., HIV and influenza virus); (iv) downregulation of expression of host major histocompatibility complex (MHC) class I molecules in infected cells, including by retention of newly synthesized class I molecules in the endoplasmic reticulum (e.g., adenovirus), by inactivation of the TAP transporter associated with antigen processing (e.g., herpes simplex virus) and by dislocation of newly synthesized class I molecules from the endoplasmic reticulum to the cytosol for degradation (e.g., cytomegalovirus) (102); (v) inhibition of antigen processing and MHC class I-restricted presentation [e.g., EBV (103)]; (vi) infection of cells deficient in MHC class I molecules (e.g., herpes simplex virus in neurons); and (vii) infection and suppression of essential immune cells (e.g., HIV). It is of interest that the majority of tumor viruses can infect lymphocytes and monocytes, although other cell types may be the primary targets for infection.

Despite these elaborate viral evasion mechanisms, the immune system frequently prevails. For example, the prevalence of HPV infections may be as high as 50% among young women, but declines with age (46,88). This suggests that the immune system renders most, but not all, infections transient. Perhaps the high-risk HPV types are relatively more efficient than other types at establishing persistent infections, contributing to their disease potential.

The hepatitis viruses linked to liver cancer are successful at avoiding immune clearance. HBV infections occurring in adults are usually resolved, but primary infections in neonates and young children tend to become chronic (78,80,104,105). Transmission of HBV to an infant from mother or siblings results in persistent infections in 80–90% of cases, presumably because the immune system is incompletely developed at birth. It is these persistent HBV infections established early in life that carry the highest risk of HCC later in life (78,80,82,106). There are currently over 250 million people worldwide persistently infected with HBV, a large pool of individuals at risk of developing a liver malignancy. With another liver-tropic virus (HCV), it appears that up to 85% of infections may become persistent, even in adults (81); it may be relevant that HCV proteins can inhibit the antiviral effect of the interferon response, an early host response to viral infection. Chronic infection with HCV is also considered to be a causative factor in HCC and it has been estimated that there are as many as

Table VII. Properties of accepted and potential human tumor viruses

Characteristic	HBV	EBV	HPV	HTLV-I	HCV	HHV-8 (KSHV)	SV40	MCV
Genome								
Nucleic acid	dsDNA[a]	dsDNA	dsDNA	ssRNA→dsDNA	ssRNA	dsDNA	dsDNA	dsDNA
Size (kb/kbp)	3.2	172	8	9.0	9.4	165	5.2	190
No. genes	4	≈90	8–10	6	9	≈90	6	≈180
Cell tropism	Hepatocytes, white blood cells	Oropharyngeal epithelial cells, B cells	Squamous epithelial cells (mucosal, cutaneous)	T cells	Hepatocytes	Vascular endothelial cells, lymphocytes	Kidney epithelial cells, others	Epidermal cells
Unique biology	May cause chronic infection and inflammation	Immortalizes B cells	Highly species and tissue specific, replication dependent on cell differentiation	Immortalizes T cells, encodes trans-acting factor	High rate of chronic infection and inflammation	Contains many cellular genes	Stimulates cell DNA synthesis	Species and tissue specific
Prevalence of infection	Chronic infections common – Asia, Africa	Common	Common	Common – Japan, Caribbean	Common	Not ubiquitous		
Transmission	Vertical, parenteral, horizontal, venereal	Saliva	Venereal, skin abrasions	Breast milk, parenteral, venereal	Parenteral, horizontal	Horizontal, venereal	Urine?	Contact, venereal
Human diseases	Hepatitis, cirrhosis	IM, oral hairy leukoplakia	Skin warts, EV, genital warts, LP	HAM/TSP	Hepatitis, cirrhosis			
Human cancers	HCC	BL,[b] NPC,[c] HD, lymphomas	Cervical, skin, oropharynx	ATL	HCC	KS, PEL, Castleman's disease	Brain, bone, mesothelioma	MC
Transforming genes	HBx ?	LMP-1	E6, E7	Tax ?	NS3?		Large T-antigen, small t-antigen	
Viral genome integrated in human tumors	Usually		Usually	Yes (provirus)	No			No

HD, Hodgkin's disease; IM, infectious mononucleosis; KS, Kaposi's sarcoma; LP, laryngeal papillomas; MC, molluscum contagiosum; PEL, primary effusion lymphoma.
[a]Partially double-stranded (ds) and partially single-stranded (ss) in virion.
[b]Equatorial Africa = endemic; elsewhere = sporadic.
[c]Southeast Asia = common.

170 million chronic carriers worldwide, ~3% of the world's population (107).

EBV is similarly successful at avoiding immune elimination, and infections with EBV are life-long. This may be explained in part by the function of EBNA1 in inhibition of antigen processing to allow infected cells to escape from cytotoxic T lymphocyte surveillance (103); EBNA1 is expressed in latently infected B cells and in all EBV-associated malignancies.

It is believed that immunocompetent hosts mount strong immune responses against virus-transformed cells and prevent tumor outgrowth. Presumably, this surveillance mechanism efficiently eliminates the rare neoplastic cells that may arise in normal individuals infected with cancer viruses. However, if the host is immunosuppressed, cancer cells may proliferate and escape host immune control. It is known that immunosuppressed organ transplant recipients and HIV-infected individuals are at increased risk of EBV-associated lymphomas (85,86,108) and of HPV chronic infections and related diseases, including HPV-positive skin cancer (46,88). These observations suggest that individuals at elevated risk of virus-associated cancer include not only organ transplant recipients and HIV-infected persons, but also the very young and patients undergoing immunosuppressive therapy for some medical condition. It is possible that unrecognized variations in individual immune responses may contribute to susceptibility to virus-induced tumors in presumably normal hosts.

These long-term complex interactions between virus and host are important features of human tumor viruses, as they set the stage for continual infections of new cells, mitogenesis, mutation, selection and/or inflammation, some or all of which may contribute to eventual virus-mediated tumorigenesis (109). Even though very few cells in the host may be infected at any given time, the chronicity of infection presents the long-term opportunity for a rare event to occur that allows survival of a cell with growth control mechanisms that are virus-modified. These virus–host interactions cannot be modeled meaningfully in tissue culture, and there is usually no available animal model that faithfully mimics the human pattern of cycles of virus replication, tissue spread and host responses.

Viruses are seldom complete carcinogens

In those cancers that have a viral etiology, the virus appears to be necessary, but not sufficient, for tumor development. The interpretation is that viruses usually do not behave as complete carcinogens, but rather act as initiating or promoting factors. Additional changes must accumulate to complement those mediated by viral functions in order to disable the multiple regulatory pathways and checkpoints in normal cells and to allow a cell to become completely transformed.

Different types of data support this interpretation. First, cancer development is not an inevitable outcome of virus infection in any viral system. The majority of individuals naturally infected with a tumor virus do not develop cancer. Whereas >90% of humans are infected with EBV, disease is rare unless the host becomes immunocompromised. Estimates are that 2–6% of those infected with HTLV-I develop ATL (89), and only a small fraction of persistent HPV infections progress to cancer. Infection with a high-risk HPV presents a woman with about a one in 30 lifetime risk of developing cervical cancer (46). At the other extreme, it has been reported that ~40% of adult Chinese males with chronic HBV infection die of cirrhosis or HCC (106) and that Japanese males have a lifetime estimated risk of developing HCC of 20% in HBV chronic carriers and 30% in HCV carriers (83).

Long latent periods are the norm between the time of initial virus infection and tumor appearance in normal individuals. Chinese with chronic HBV infections acquired as newborns usually develop HCC beyond 50 years of age (78,80), there may be >30 years between EBV infection and development of NPC or Hodgkin's disease (108), 3–4 decades typically elapse between HPV infection and development of cervical cancer (46,60) and, although most HTLV-I infections are acquired in infancy, ATL usually arises in people in their forties and fifties (90).

Viral strains. HPV is the paramount example of the existence of viral strains differing in oncogenic potential (46,60,87). More than 75 different types of HPV have been described, based on genotyping by DNA sequence analysis rather than standard serotyping as serologic tests do not exist for these viruses, and about 30 of these types can infect the genital tract. The distribution of HPV genital types detected changes with increasing severity of cervical disease (110), as only a few types are detected in cervical carcinomas out of the plethora of virus types causing infections in normal women. The HPV types found in invasive cancers are designated 'high-risk' HPVs because they are associated with lesions at high risk for malignant progression. High-risk HPV DNA can be detected in >90% of cervical carcinomas, with HPV-16 accounting for over half of the cases, followed by HPV-18, HPV-45 and HPV-31 (46,87,88,111). There is no evidence of such pronounced diversity in oncogenic properties among strains of other human tumor viruses, although HCV isolates show extensive genetic heterogeneity (112), and there are suggestions of strain variation in the EBV oncogene, LMP1 (85). There is the precedent with polyoma virus in mice that strains may differ profoundly in their abilities to induce numbers and types of tumors *in vivo*, yet are equally efficient at transformation of cells *in vitro* (113). The theoretical possibility exists that unrecognized viral strain differences may be partially responsible for the relatively infrequent cancers that occur among persons infected with a cancer-inducing virus.

Cofactors. Various endogenous and exogenous agents have been investigated as possible cooperating components in virus-mediated carcinogenesis. Different cofactor elements have been linked with virus-related human tumors, and it appears that the cofactor influence may vary geographically. Endemic BL occurs with high incidence in children in equatorial Africa coincident with high endemic malarial infections, suggesting malaria as a contributing factor, perhaps due to an effect on the immune system. Most BL that occur in these regions are EBV-positive and also show characteristic chromosomal translocations between the c-*myc* gene and immunoglobulin loci, leading to the constitutive activation of *myc* expression (28). Long-term consumption of salted or dried fish, known to contain high levels of nitrosamines, has been implicated as a dietary cofactor in EBV-related NPC that occurs frequently in Cantonese Chinese and in Alaskan Eskimos (84). Dietary exposure to another carcinogen, aflatoxin, has been proposed as a cofactor for HCC, especially in west Africa, Mozambique and southern China (78,114). Exactly how dietary carcinogens cooperate with viral infection to increase cancer development is not known. However, it has been concluded that selected groups of individuals are likely to suffer greater risk from exposure to environmental carcinogens than the general population (115).

Although controversial, a molecular basis for a cofactor effect of the host genetic background on viral carcinogenesis involving HPV has recently been described (116). The human *p53* gene is polymorphic at codon 72, encoding either a proline or an arginine. p53 is targeted for degradation by the E6 protein of high-risk types of HPV, and it was shown that the arginine form of p53 is much more susceptible to degradation by E6 than the proline form. Patients with cervical cancer reportedly had an overrepresentation of homozygosity of the p53 arginine allele compared with the normal population. Presumably, polymorphisms in other human genes could provide similar predispositions to virus-induced cancer formation.

Patients with the rare skin disease EV represent a natural example of a mixture of genetic, viral and environmental factors contributing to cancer development (46). EV is a life-long disease beginning early in life, characterized by disseminated skin lesions that are associated with HPVs (more than 20 different types). About 50% of EV patients develop skin cancers in their twenties and thirties; the cancers usually occur in sun-exposed areas of the body, suggesting that ultraviolet radiation is a cofactor. Only a few of the many EV-associated HPVs have been connected with progression of EV lesions to malignancy (types 5 and 8). Finally, the majority of EV patients have impaired cell-mediated immunity, presumably reflected in a weakened host response to HPV infection that contributes to their disease.

Mechanisms of action of human cancer viruses

Human tumor viruses display different mechanisms of cell transformation and fall into both direct- and indirect-acting categories. Direct-acting viruses carry one or more viral oncogenes, whereas the indirect-acting agents appear not to possess an oncogene. The latter agents, in contrast to the former, generally lack demonstrable transforming activity in *in vitro* assays. The diversity of oncogenic mechanisms by human tumor viruses emphasizes that there is no single mode of transformation underlying viral carcinogenesis. As described above, even the direct-acting viruses are not complete carcinogens. The proven human cancer viruses are all replication competent and establish long-term persistent infections in various cell types; the occasional destructive outcome, cancer, is an accidental side-effect of viral replication strategies.

Direct-acting tumor viruses. DNA virus oncoproteins are viral replication proteins; their purpose in the life cycle of the smallest tumor viruses is to direct the infected cell into a physiological state supportive of virus gene expression and replication, as the viruses do not encode enzymes necessary for DNA replication. The direct-acting papillomaviruses and polyomaviruses encode oncoproteins that target cellular tumor suppressor proteins (Table IV). As described above, inactivation of the p53 and pRb tumor suppressor proteins in cells that are not killed by virus infection is central to cell transformation by the small DNA tumor viruses.

In addition to the E6 and E7 transforming proteins, the papillomaviruses also encode an early protein designated E5. The bovine papillomavirus can transform fibroblasts and E5 is the functional oncoprotein in those cells. The E5 protein complexes with the platelet-derived growth factor β receptor and activates it in a ligand-independent fashion to mediate a sustained mitogenic signal (117). It is not yet clear whether the E5 proteins of HPV modulate signal transduction pathways in epithelial cells, analogous to the bovine papillomavirus E5 effect in fibroblasts.

EBV is another direct-acting tumor virus; it encodes a viral oncogene protein (LMP1) that resembles a cell-surface receptor. That protein is able to transform rodent fibroblasts, is essential for transformation of B lymphocytes, and is expressed during the lytic cycle. LMP1 mimics an activated growth factor receptor and interacts with tumor necrosis factor receptor-associated factors (TRAFs) that associate with activated receptor and mediate its proliferative signals (118). LMP1 activates the NF-κB transcription factor in B lymphocytes and modulates the epidermal growth factor receptor in epithelial cells, probably due to its binding of TRAFs (85,117). Several of the EBV-encoded nuclear antigens (EBNAs) are necessary for immortalization of B cells. EBNA1, the only viral protein consistently expressed in BL cells, has been shown to be oncogenic in transgenic mice (119).

Indirect-acting tumor viruses. The indirect mechanisms of tumor induction defined by the non-transforming animal retroviruses, such as promoter insertion (described above), do not appear to be common in virus-induced human cancer. The production of HCC following HBV infection probably involves a combination of indirect mechanisms, including an immune response component, together with more direct mechanisms that cannot be measured easily in cell cultures. Chronic liver injury secondary to persistent viral infection leads to necrosis, inflammation and liver regeneration which, over many years, results in cirrhosis; HCC usually arises out of this background (79). Attention has focused on the HBV transactivator protein, the X protein, as the potential viral oncoprotein. The X protein appears not to be a directly-transforming gene analogous to the HPV and EBV viral oncogenes; it probably contributes indirectly to the process of liver carcinogenesis by activating the Ras-Raf-mitogen-activated protein kinase signalling cascade (120) and transactivation of cellular proliferation-related genes, and/or by hampering the function of the cellular DNA repair system and allowing mutations in cell genes to be propagated (121–123). It is presumed that the mechanistic role of HCV in the development of HCC is similarly indirect, i.e. by the induction of chronic hepatocellular injury, coupled with inflammation and liver cell regeneration. This is a plausible hypothesis as the HCV genome resembles that of the flaviviruses, consisting of single-stranded RNA and having no evidence of integration of viral genes into the cellular genome. HCV does not carry a classical oncogene but it has been reported that viral non-structural protein NS3 can transform NIH 3T3 cells and can bind p53 (124,125).

The hyperplastic growths and skin tumors induced by poxviruses are another example of a cellular response to a virus effect on a signalling cascade. The poxvirus proliferative responses might be explained by the secretion from infected cells of virus-coded growth factors that are related to epidermal growth factor (126), with subsequent stimulatory effects on neighboring cells. By induction of localized hyperplasia, more metabolically active cells would become available for virus infection. It is of interest that the poxvirus growth factors are much more biologically potent than their mammalian counterparts.

The vicious circle of events generated by some persistent viral infections can be envisioned as a wheel that keeps turning, powered by the chronic infection and the attendant host responses (Figure 5). The speed of rotation would be determined by a combination of factors, including the degree of virus replication, the virulence of the infecting virus,

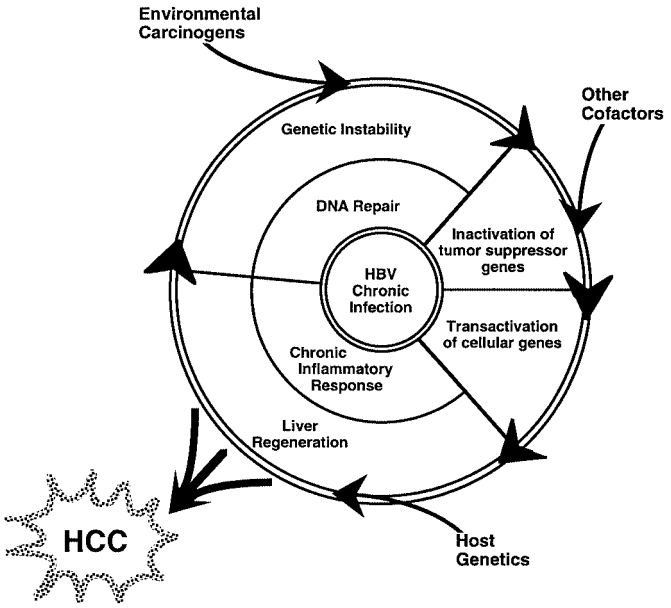

Fig. 5. Model for the role of HBV in the development of HCC. Chronic infection by HBV is central to the process. The resulting inflammatory response and liver regeneration introduce the potential for errors during DNA replication. The HBV X protein interacts with DDB1, a cellular DNA repair protein. If viral infection were to reduce the ability of the cell to repair damaged DNA, mutations would accumulate over time, increasing the likelihood that tumor suppressor genes such as *p53* would get functionally inactivated. The X protein can *trans*-activate cellular genes; affected cell genes might contribute to carcinogenesis. Environmental carcinogens are important cofactors in certain areas of the world and would be especially potent if viral infection had crippled the cellular DNA repair system. Host genetics influences the host response to HBV infection. When the appropriate genetic changes accumulate over many years, HCC occurs. Reproduced, with permission, from Butel *et al.* (121).

characteristics of the individual's immune response, and the possible presence of environmental cofactors. The eventual outcome in the case of chronic HBV infection may be HCC. Aspects of this model can be extrapolated, in principle, to other persistent viral infections that lead to development of human cancer.

HTLV-I is currently the only retrovirus accepted as having an etiologic role in a specific human cancer. It appears to act indirectly in the development of ATL. The viral Tax protein has transcriptional activation properties and is presumed to provide a trans-acting function in the transformation process. It has been reported that Tax binds to MEKK1 protein kinase and activates NF-κB (127); there are also recent indications that Tax may exert deleterious effects on the DNA repair system of the cell (128,129), similar to what has been shown for HBV. The role of HIV in carcinogenesis is probably even more indirect (130). Immunosuppression secondary to HIV infection predisposes those individuals to certain cancers, especially EBV-positive lymphomas, HHV-8(KSHV)-positive KS and HPV-positive tumors. In addition to this indirect effect, a more direct role for HIV in the genesis of KS has been proposed (131), involving a cellular growth-promoting effect by the HIV-1 tat protein. Much remains to be deciphered about the role of these retroviruses in human neoplasia.

Viruses and cancer: passengers or perpetrators?

There are many difficulties encountered in attempts to establish an etiologic role for a virus in human cancer, explaining why laborious and sustained efforts are necessary to prove a causal association. The problems in establishing viral etiologies (Table VIII) have been described by Evans and Mueller (132) and by zur Hausen (60). Several of these difficulties reflect the basic nature of viral infections, discussed above. One obstacle to be overcome is that the mere presence of a virus in a tumor does not prove etiology. The 'passenger virus' dilemma is pronounced when the host tissue containing the tumor is the normal site of virus replication. Other explanations to consider are that the virus might have infected the tumor following tumor outgrowth because the proliferating tumor cells provided a favorable environment for virus replication; the virus might be present in non-tumorous cells, such as lymphocytes, intermingled within the tumor; or the sample might have been inadvertently contaminated with the virus in the laboratory. Demonstration of viral nucleic acid and gene expression within the actual tumor cells resolves issues of which cells in a neoplasm are virus-infected. Good laboratory practices and inclusion of appropriate controls can eliminate the concern about laboratory contamination. However, technical difficulties may be impediments in establishing an etiologic role in some systems: there may be no easy assays to detect the virus in tumors, serology may be uninformative with respect to the prevalence of infections by the virus, and there may be no animal model to confirm the suspected oncogenic potential of the virus. In addition, common characteristics of virus-induced tumors present other problems. Virus replication often does not occur in tumor cells, and the entire viral genome is frequently not retained, limiting the assays able to detect the presence of virus. Due to tumor progression and heterogeneity, it is possible that, by the time tumors are recognized and studied, the viral transforming genes involved in tumor initiation may have become redundant and lost from some tumor cells.

Table VIII. Difficulties in establishing an etiologic role for a virus in human cancer[a]

1.	Virus may be ubiquitous in humans, but cancer is rare
2.	Long time interval usually occurs between virus infection and tumor appearance
3.	Time of initial viral infection may be unknown
4.	Host factors that are important determinants in susceptibility to cancer will vary
5.	Viral strains may differ in biologic properties, including oncogenic potential
6.	Virus-related carcinogenesis may require cofactors
7.	Chemical and physical carcinogens may also be related to same type of cancer
8.	Causes of cancer may vary in different geographic areas or in different age groups
9.	Available assays for detection of virus infection may be uninformative
10.	No animal model may exist

[a]Modified from Evans and Mueller (132) and zur Hausen (60).

Reflecting these difficulties, the etiologic role of a virus in a human cancer is usually slow to be accepted, with a decade or more sometimes elapsing before general agreement is reached of a causative association. Despite these hurdles, several viruses have become accepted as etiologic factors in human malignancies, and several others are likely candidates to join that company. Today, the power of modern molecular biology is facilitating the accumulation of virologic data from human samples. The sensitivity and specificity of the polymerase chain reaction (PCR) and nucleic acid sequencing

techniques avoid many of the ambiguities of older techniques that relied on detection of infectious virus or were unable to detect low-abundance viral markers in human tumors, sometimes leaving doubts as to which precise agent was present (24).

Different routes to evidence of causality

Evans and Mueller guidelines. After considering the difficulties of proving a causal link between a candidate tumor virus and a human cancer (Table VIII), Evans and Mueller (132) suggested types of evidence that would support an etiologic role for a virus. Suggested epidemiologic guidelines included: (i) the geographic distribution of viral infection should coincide with that of the tumor, adjusting for the presence of known cofactors; (ii) the presence of viral markers should be higher in case subjects than in matched control subjects; (iii) viral markers should precede the tumor, with a higher incidence of tumors in persons with the marker than in those without; and (iv) prevention of viral infection should decrease tumor incidence. Suggested virologic guidelines included: (i) the virus should be able to transform human cells *in vitro*; (ii) the viral genome should be demonstrated in tumor cells and not in normal cells; and (iii) the virus should be able to induce the tumor in an experimental animal. Some of these guidelines are very difficult to achieve (e.g., the only practical prevention modality available for the recognized human tumor viruses is a vaccine for HBV), and some guidelines are not applicable to all virus systems (e.g., as many human cancer viruses are ubiquitous and establish persistent infections, control subjects will be infected, and normal cells in tumor-bearing individuals may be infected). The guidelines are very useful, however, in helping investigators evaluate evidence of a putative viral association with human cancer.

Hill criteria for causality. Sir Austin Bradford Hill (133,134) proposed general epidemiologic criteria to establish causation between a disease and an environmental factor (infectious or non-infectious). The Hill causal criteria are: (i) strength of association (e.g., how frequently is the virus found in the tumor?); (ii) consistency (has the association been observed repeatedly by different people in different places?); (iii) specificity of association (is the virus, or specific variants of the virus, uniquely associated with the tumor?); (iv) temporal relationship association (does virus infection precede tumor development?); (v) biologic gradient (is there a dose–response relationship with virus load?); (vi) biologic plausability (is it biologically feasible that the virus could cause the tumor?); (vii) coherence (does the association make sense with what is known about the natural history and biology of the disease?); and (viii) experimental evidence (are there supporting laboratory results?). The Hill criteria for causality are not absolute, considering the complexity of virus–host interactions and the multiplicity of cancer-causing factors, but do provide helpful guidelines for assessing a possible virus–cancer linkage. The most important criterion is that the correct temporal relationship must occur. However, proof of causality requires virologic data, as well as epidemiologic observations, and the modern advances in molecular biology are changing the strength and type of data proof that can be obtained.

With these guidelines as background, it is instructive to briefly review some examples of different approaches that were taken to develop viral links to human cancers.

EBV. Seroepidemiology was important in establishing a role for EBV in BL, after EBV particles were detected in BL-derived tumor cell lines by electron microscopy and prior to the advent of modern molecular technology. Although the virus is distributed worldwide and most adults have antibodies, both BL patients and NPC patients had much elevated titers of antibodies to EBV viral antigens, suggesting an altered virus–host interaction, and prospective epidemiologic studies showed that high antibody titers preceded the development of BL (135). Virologic studies years later confirmed the linkage. These data included detection of EBV DNA and analysis of selective EBV gene expression in tumor cells, recognition of EBV latency in B cells, and identification and characterization of EBV immortalizing and transforming genes. More recent studies using mainly viral assays have established associations of EBV with Hodgkin's disease and other lymphomas.

HBV. The known population distributions of chronic infection by HBV and of occurrence of HCC led to recognition of a strong association between the two (106). This association was subsequently confirmed in different populations in other regions of the world. Molecular studies detected viral markers in tumors, but the mechanism of HBV involvement in liver carcinogenesis remains the subject of investigation today.

HCV. Once HCV was detected and cloned after an intensive search for non-A, non-B hepatitis viruses in infectious sera of people with post-transfusion hepatitis, its role in HCC was investigated, as not all HCC had been found to contain HBV markers (81). Using a combination of serologic and molecular assays developed soon after HCV was cloned, a strong association was observed between HCV infection and risk of HCC. The available evidence is based largely on epidemiologic studies, as there are no tissue culture systems or animal models for HCV infections. It is noteworthy that an association of HCV with HCC was sought (and confirmed), despite the fact HCV was determined to be a flavivirus, a type of virus not suspected of being capable of tumorigenic activity. The molecular mechanism of HCV oncogenicity remains to be established.

HPV. The proven tumorigenicity in animals of rabbit and bovine papillomaviruses, coupled with epidemiologic suggestions of venereal transmission of an infectious agent involved in cervical cancer and the molecular detection of papillomavirus DNAs in various human lesions, prompted the hypothesis of HPV involvement in genital cancer (136). Follow-up studies using recombinant technology revealed the existence of a multiplicity of HPV types and the association of a subset of specific viral types with cancers (137). New virus strains were identified originally using low-stringency hybridization probes and, more recently, by PCR with degenerate primers. The epidemiologic data on HPV and cervical cancer have fulfilled the Hill criteria of causality (88), and there is mounting evidence that HPVs are also associated with other cancers, including those of the skin, the oropharynx and other anogenital sites. It is clear that the HPVs are major cancer pathogens in humans. This relationship was established without the use of seroepidemiology, which played a major role in establishing several other virus–cancer connections, because the serologic relatedness of the many HPV types has not been determined and many cervical cancer patients with HPV-positive tumors do not have detectable immune responses to HPV antigens. Subsequent laboratory studies have identified the viral transforming genes and their mechanisms of action, the general molecular biology of the viruses, and the differing biologies of the high- and low-risk types. It has been established that HPV viral genomes are maintained as episomes in benign

lesions, but are generally found integrated in the chromosomal DNA of cancer cells, although up to one-third of cervical biopsies may contain only episomal DNA. The integration of the viral DNA retains the integrity of the E6 and E7 transforming genes, but often disrupts the E2 gene, resulting in elevated expression of E6 and E7 proteins (46,87).

HHV-8(KSHV). Based on hints from epidemiologic studies that KS might be caused by an infectious agent (92), modern molecular technology [representational difference analysis (RDA)] was used and successfully identified sequences of a new herpesvirus [HHV-8(KSHV)] in a KS skin lesion (76). This approach was undertaken to detect unique sequences present in tumor cells as compared with normal cells, without knowing the nature of the putative agent. The virus [HHV-8(KSHV)] is very difficult to culture (138), but seroepidemiology and molecular assays have been used to study the relationship of the virus to human disease and those findings strongly associate this newly recognized herpesvirus as a causative factor of KS (91,92,97). Its mechanistic role in disease development is still unclear.

Polyomaviruses. Finally, knowledge of the biology of the polyomaviruses, potent tumor viruses under experimental conditions, prompted a search for viral sequences in the same types of human tumors as those induced in laboratory animals. Although there have been reports of detection of DNA sequences of human polyomaviruses JCV and BK virus (BKV) in human tumors (139), it is the DNA of SV40 that has been reproducibly detected in certain tumor types (24,140,141). These observations were unexpected and were met with some scepticism because SV40 was assumed to be a monkey virus that did not infect humans. However, molecular approaches have confirmed that authentic SV40 was detected and previously unrecognized sequence variability indicative of strain variation allayed concerns of possible laboratory contamination of tumor samples. The tumor types induced in rodents by SV40 have predicted the major types of human tumors found to contain SV40 DNA—brain tumors, osteosarcomas and mesotheliomas—and the expression of T-ag has been demonstrated in some tumor cells. Virologic and biologic data are strongly suggestive of a role for SV40 in certain types of tumors, but epidemiologic data of the distribution and prevalence of human infections are lacking (24). It is curious that JCV and BKV have not been linked with specific human tumors, although they are also oncogenic in laboratory studies. Perhaps SV40 is a recent acquisition by humans and is less well-adapted and more pathogenic to humans. It is also possible that some basic difference between the T-antigens of the polyomaviruses makes SV40 in humans the equivalent of a high-risk HPV, whereas JCV and BKV are similar to low-risk types (24). Much remains to be learned of the role of polyomaviruses in human disease.

Importance of animal models

The value of animal models to progress in viral carcinogenesis cannot be overstated, especially with respect to investigating mechanisms of transformation. Several examples of their contributions to viral studies will be noted for illustrative purposes. However, the animal models have been limited so far in their ability to mimic the pathogenesis of virus-induced cancer in humans.

Sources of tumor viruses and proof of oncogenicity. The collection of cancer-causing retroviruses recovered from spontaneous tumors in animals, especially from chickens and mice, provided the tools for dissecting the biologic and molecular mechanisms of carcinogenesis (1). These studies depended primarily on agents that caused disease in their natural hosts. The acutely-transforming retroviruses yielded the direct-acting oncogenes that revealed the role of abnormal cellular genes in human cancers, including those of non-viral origin. The non-transforming retroviruses that induce tumors *in vivo* after a long latent period revealed indirect mechanisms of carcinogenesis, including cellular proto-oncogene activation by proviral insertion of promoter and enhancer sequences (described above). Endogenous retroviruses were recognized as resident sequences in the cellular genome, including in the human genome; these occur as both complete or defective genomes and in expressed or silent forms. Surprisingly, endogenous MMTV genomes were found to be the source of superantigen genes in mice, the expression of which control the Vβ gene families expressed by T cells in different strains of mice (15,19), illustrating that endogenous retroviral sequences can have functional effects on the host.

Many of the DNA tumor viruses were recognized to have oncogenic properties by injection of virus into experimental animals. These cross-species approaches were necessary because tumors in the natural hosts are rare, if they are observed at all, and typically have long latent periods. Prime examples are the studies of polyomaviruses and adenoviruses in rats, mice and hamsters (24,139,142). These systems revealed the existence of viral oncoproteins, provided the means of analyzing viral transforming properties, produced essential immunologic reagents that were used to detect viral proteins in cultured cells, and led eventually to recognition of the importance of tumor suppressor proteins in cancer development (described above). Rodent models have also supported studies of the host immune response to virus-induced cancer cells, including both antibody and cytotoxic T lymphocyte responses. Studies in mice have shown that immune responses against the SV40 viral oncoprotein can protect the host from the outgrowth of SV40 tumor cells (143–145), providing a precedent for the concept of vaccinating humans against viral antigens expressed in cancer cells.

Transgenic mice. The development of transgenic mice added a new dimension to studies of viral oncogenes (50,53,146,147). Individual viral genes could be examined for effects on different cell types by using tissue-specific promoters, combinations of transforming genes could be compared and mutant genes could be tested to dissect viral transforming functions. It has been $<$20 years since the first demonstration that SV40 under the control of its natural regulatory region induces brain tumors (choroid plexus type) in transgenic mice (148), significant because human brain tumors have been found to contain SV40 DNA. It has since been established that SV40 T-ag is a potent oncoprotein and is able to induce neoplasms in a variety of tissues in transgenic mice, the affected tissue being determined by the transcriptional controls governing T-ag expression. A pattern emerged from various transgenic models, including those involving T-ag: oncogene expression caused the development of widespread proliferative hyperplasias, followed by the appearance of a few distinct tumor foci, suggesting that additional changes beyond oncogene expression were required for tumor formation (50). Oncogene cooperativity was demonstrated by crossing transgenic animals carrying different transgenes (such as c-*myc* + H-*ras*, c-*myc* + v-*abl* and *Wnt-1* + *Int2*); doubly transgenic progeny exhibited accelerated tumor

onset (52,53,146,147). SV40 T-ag transgenic mice showed that transformed cells may lose their dependence on the initiating viral oncoprotein over time (51,149) and that T-ag transforming functions required for tumor development differ among cell types [e.g., the p53-binding domain is not essential for induction of choroid plexus tumors (146)]. Transgenic mice studies were important in establishing the roles of HPV E6 and E7 in tumor development and in studying differences between high- and low-risk virus types (46,147,150).

Transgenic mice carrying HBV genes have revealed the importance of inflammation associated with chronic virus production in the development of liver cancer (151). Overexpression and accumulation of the surface antigen of HBV in hepatocytes resulted in liver injury, inflammation and liver regeneration. Different lineages of transgenic mice exhibited different degrees of protein accumulation and liver damage and the development of HCC corresponded directly to the extent of liver injury. This model supports the concept that indirect mechanisms of carcinogenesis may be related to host responses to persistent viral infections. These types of observations provide a clearer understanding of the variety of mechanisms of carcinogenesis possible in human tumors.

Knock-out mice. The breadth of carcinogenesis studies was expanded when gene-targeting technologies made it possible to mutagenize specific genes in the mouse germ line. 'Knock-out' mice have been used to study the function in development, cell differentiation and carcinogenesis of cellular proteins that had been identified as targets of viral oncoproteins (54,55). Tumor suppressor genes are known to be necessary for proper cell growth control and their functional inactivation was shown to be important in DNA tumor virus transformation. The *p53* gene was the first tumor suppressor gene to be disrupted in the mouse (152,153). Homozygous p53-null mice develop normally, but are subject to spontaneous tumors in multiple tissues at an early age. Heterozygous animals have an increased cancer susceptibility at later ages and, surprisingly, the spectrum of spontaneous tumor types that develop differs from that in the p53-null animals and is influenced by the genetic background of the host. The p53-deficient mice have been used to examine the role of p53 in tumor progression, in combination with viral and cellular oncogenes, and in response to other classes of carcinogens (54,55). For example, p53 deficiency was shown to accelerate mammary tumorigenesis in *Wnt-1* transgenic animals (154) apparently via increased cell proliferation and genetic instability.

Tumor cell lines. Tumors and tumor cell lines from animal models, as well as cells transformed in culture, have provided the materials for studies of retention and expression of viral markers in tumor cells. There are generally viral markers present in virus-induced tumors. In retrovirus-induced tumors, the viral provirus is integrated in the chromosomal DNA of all the tumor cells. In rodent tumors induced by injection of a polyomavirus, the viral DNA is retained and is usually integrated, but episomal viral DNA molecules can be detected (139). Episomal SV40 genomes have been found in human transformed cells as well (155). The entire viral genome is often not retained, as viral replication is not required for maintenance of the tumor, but the genes encoding the viral oncoproteins are normally present and the expression of the transforming proteins can be detected in most cells. However, loss of T-ag has been reported from neuroectodermal rat tumor cell lines (156), and other exceptions to the generalization were described above.

Limitations. Unfortunately, the available small animal models have major limitations as surrogates of virus-induced cancer in humans. If the virus is unable to replicate in the laboratory animals (e.g., SV40), the dynamics of persistent infections cannot be modeled, including the possibility of evolution of virus variants, the infection of different cell types and the influence of host responses. In such instances, the model will not reflect all aspects of the virus–host relationship characteristic of a natural infection. In other cases (e.g., EBV, HTLV-I), there is no useful animal model.

Opportunities and future directions

It is difficult to predict what the future holds for viral oncology, as no one could have foreseen the revolutionary advances of the last 20 years that emanated from virus studies. However, several areas of opportunity and avenues of investigation seem likely to keep viral carcinogenesis at the forefront of cancer studies in the new millenium.

Molecular mechanisms of viral oncogenesis

The general patterns are now established of how transforming genes function and collaborate, including cellular oncogenes, tumor suppressor genes and viral oncogenes. This outline will continue to be fine-tuned and more molecular details and variations added in the future. Viruses are the ultimate model systems for unraveling molecular and cellular mechanisms, and just as tumor viruses shaped modern cancer biology, it is expected that viral systems will prove equally useful in the future. We can anticipate the development of targeted interventions based on the biochemical pathways disrupted by oncoproteins and on new knowledge of the structural basis of molecular interactions. Practically any gene or protein that plays a significant role in carcinogenesis is a legitimate therapeutic target. Many new drugs are under development, such as antibodies that block the activity of cell surface receptors and small-molecule drugs that block oncogene growth signal transmission inside the cell. The hope is that such new therapies will be more specific to cancer cells than current chemotherapies and less harmful to normal cells. The choice of therapy for a cancer patient may one day be guided by the molecular fingerprints of a given tumor.

Studies will continue to characterize the molecular mechanisms of viral involvement in human cancers. The precise roles played by viruses in those human cancers with viral cofactors remain puzzling, and unraveling the molecular complexities of viral contributions is a serious challenge for the future. More attention needs to be focused on early steps in tumorigenesis. As studies of late-stage tumors cannot reveal the initiating early events, analyses of early-stage lesions are necessary in order to learn how a rare rogue cell is able to escape host control and progress into a recognizable tumor. It is possible that, on occasion, viral gene functions involved in the early stages of tumorigenesis may become redundant as growing tumor cells become more autonomous, and the viral genes may be lost from advanced tumors. Additional indirect mechanisms of action by human tumor viruses will probably be realized. There are the current indications that viral infections may affect the cellular DNA repair system, supposedly allowing the accumulation of mutations in growth regulatory genes, or that processes and reactants associated with a virus-induced inflammatory response may predispose to cancer. It is possible that other normal mechanisms of host homeostasis and response to infection, under the pressure of chronic viral replication,

can go awry and promote tumor outgrowth. Finally, the hit-and-run mechanism of viral involvement in carcinogenesis should be considered as a possibility. The concept that a virus can initiate the transformation process through a mutagenic mechanism and then disappear without leaving viral traces has fallen out of favor. As more examples of indirect mechanisms of viral involvement in human tumors are found, this discarded theory of viral action should be remembered and evaluated.

More human cancer viruses

It is now firmly established that viruses cause human cancer. It is expected that studies of possible viral involvement in human preneoplasia and neoplasia will be continued and that new associations will be found. This may include the discovery of new agents as well as the identification of unsuspected tumor associations involving agents that are already known. Human leukemias and lymphomas are prime tumor types for possible viral involvement, considering the many precedents in animal models. The small DNA tumor viruses, the polyomaviruses, deserve special attention as potential human cancer agents. There are accumulating reports of an association of SV40 with human tumors (24,93), and there have been several reports of detection of JCV and BKV sequences in human tumors (139). It has recently been proposed that polyomaviruses, especially JCV, are candidates for etiologic roles in some cases of childhood leukemia (157). As infectious virus is often not retained in tumors, searches for viral associations with different tumor types will rest on the application of molecular methods that are not dependent on the presence of intact virus in test samples, such as PCR, RDA and new innovative approaches that will undoubtedly be developed. The unexpected addition of a flavivirus (HCV) to the ranks of human tumor viruses opens the possibility that other less-standard viral cofactors may be involved in cancer development. What is critical is the biology of the virus and the nature of the virus–host interaction, not our current views of how a tumor virus mediates tumorigenesis.

Once new pathogens are recognized, the long process of assessing causality will be undertaken (95). Among the attendant challenges will be determining the genetics and biology of tumor-associated strains of virus, the details of virus–tumor cell interactions, the geographic distribution and age relationship of virus-associated tumors and the epidemiology of human infections by the candidate human cancer virus. Evidence of possible virus–cancer linkages should be pursued vigorously as it is of potential benefit to the public and those suffering from disease if the historically long delays of acceptance of a viral role in human cancer can be shortened. During such confirmatory testing, it is to be expected that there will be conflicting reports of successes and failures of finding virus–tumor associations until the variables affecting a particular virus–cancer relationship are identified.

More attention should be paid to determining if genetic differences among viral strains impact the development of virus-associated cancer. Lessons from the human papillomaviruses emphasize the biologic importance of viral strain differences. Identification of genetic markers of tumorigenic strains of virus would be important medically and would contribute toward an ability to predict cancer risk among infected persons.

Cancer susceptibility and host responses

An area of relative ignorance that deserves more attention is the basis of what determines host susceptibility to cancer. Inherited mutations in tumor suppressor genes, such as p53 mutations in families with the Li–Fraumeni syndrome (158), have been established as predisposing such individuals to cancer development. In the case of virus-induced cancer, susceptibility will probably be found to involve a variety of host factors, such as immune response genes, virus receptor genes, or host response mechanisms to environmental cocarcinogens. It is essential to gain better insights into the subtleties of the molecular pathogenesis of persistent virus infections and the influence of individual host responses, as these are integral to understanding viral carcinogenesis. Circumstances allowing more robust virus replication would lead to increased viral loads, which in turn could facilitate virus spread within the host, enhancing the opportunity for detrimental virus–cell interactions. Basic virology studies have much to contribute in this area. The interplay of viral and host proteins, some of which benefit virus replication, some of which inhibit replication and some of which counteract the effects of others, hold clues that, when revealed, can be applied to viral carcinogenesis. Host responses to viral infections are both non-specific (e.g., interferons, induction of apoptosis) and highly specific (e.g., antibodies, cytotoxic T cells); improved understanding of these responses will reveal protective processes that may falter during carcinogenesis. Ultimately, it should be possible to design rational immunotherapies directed against infected cells and tumor-associated viral antigens. Adoptive transfer of EBV-reactive T-lymphocytes shows promise as treatment for EBV-related lymphoproliferative disease (159).

More information needs to be learned about environmental cofactors that synergize with viral infections to stimulate tumor production. Both the identification of carcinogenic cofactors in specific virus systems and studies of mechanisms of action of such factors in target tissues warrant investigation. Guiding examples are the probable role of aflatoxin in cooperation with HBV chronic infections on induction of HCC in certain geographic areas (78,114) and the involvement of dietary nitrosamines in EBV-induced NPC (84). An example of a conceivable synergism that bears evaluation is the possible combinatorial effects of asbestos exposure and SV40 infection on the development of mesotheliomas (160,161).

Virus vaccines for cancer control

Recognition of a viral etiology for a human cancer provides the opportunity and rationale to develop preventive measures to inhibit virus infection and thus reduce cancer risk. Vaccines are the most effective preventive approach against viral infections, and vaccines against cancer viruses have the potential of reducing the global cancer rate. Both prophylactic and therapeutic vaccine strategies can be considered. Prophylactic vaccines induce antibodies that are able to neutralize a virus before it infects a cell and establishes an infection; therapeutic vaccines are designed to reduce or eradicate an existing infection or disease. The HBV vaccine has been used for >15 years to prevent transmission of virus to newborns and establishment of life-long persistent infections. Thirty years from now it will be clear if the use of the vaccine reduced the incidence of HCC in adults as it has done in children between 1984 and 1994 (162).

Because of the worldwide burden of HPV-related disease, papillomavirus vaccines are under development. The absence of an effective culture system for those viruses necessitates the approach of using recombinant virus-like particles (VLPs)

for prophylactic vaccine development (163,164). The HPV capsid proteins, when expressed at high levels, self-assemble into structures resembling authentic virions. The VLPs lack DNA and are immunogenic. In both the canine and rabbit model systems, inoculation with VLPs induced protection against challenge virus. Therapeutic vaccines targeted against HPV oncoproteins E6 and E7 are also under development. The prospect is that immunization with those tumor-specific antigens might improve cellular immune responses against cancer cells expressing the oncoproteins. It is anticipated that some form of HPV vaccine will be introduced for use in the foreseeable future. Vaccines against other viruses involved in human cancer should be pursued. Even if cofactors in addition to viral functions are necessary for tumor development, prevention of infection by the virus would greatly reduce the overall frequency of carcinogenesis.

Viruses as tools in cancer treatment

In addition to the new generation of cancer drugs under development based on the knowledge of altered growth regulation in cancer cells, viruses can be harnessed for novel approaches to cancer therapy. Numerous gene-based therapies targeted to malignant disease are in development or clinical trial (165). Some gene therapeutic strategies are designed to augment conventional cancer chemotherapy or immunotherapy. Viral vectors are being used to deliver tumor suppressor gene replacements (*p53*), immune response genes (cytokines), drug resistance genes (for protection of normal cells), drug sensitivity genes (for selective activation of prodrugs so they become effective locally) and genes to inhibit activated oncogenes (antisense sequences). Viral vectors used for such treatments still require further development, as those currently available all suffer from certain limitations. The future will surely see some of these therapeutic modalities achieve clinical usefulness.

Another envisioned approach is to target a lytic virus selectively to cancer cells to kill them while sparing normal cells. A prototype is being tested: a mutant form of adenovirus that fails to express E1B that can replicate in and destroy cancer cells that lack p53 (166). As ~50% of human tumors lack p53, such a virus might be a potent cytotoxic agent effective against many types of tumors. Whether or not this particular virus turns out to be clinically efficacious, the principle it embodies is worth future consideration.

To quote Aristotle, the ancient Greek philosopher, scientist and writer, 'In all things of nature there is something of the marvelous.' The future holds the promise that studies of viral carcinogenesis will yield more surprises and continued advancement in our understanding of the marvelous things of nature.

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