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POLYOMAVIRUSES AND HUMAN TUMORS: A BRIEF REVIEW OF CURRENT CONCEPTS AND INTERPRETATIONS

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1. ABSTRACT

Polyomaviruses are small DNA viruses that typically establish persistent but inapparent infections of their natural hosts, although cytolytic disease may develop if the host becomes immunocompromised. Most polyomaviruses have the ability to induce tumor formation when introduced into certain foreign hosts and are considered oncoviruses. Some polyomaviruses, including those that infect humans, have occasionally been detected in cancerous tissue of their natural hosts. This article briefly reviews the biology of polyomaviruses and explores issues pertaining to the significance of association of polyomaviruses with human tumors.

2. CLASSIFICATION OF POLYOMAVIRUSES

The Papovaviridae family is comprised of two genera: *Polyomavirus* and *Papillomavirus*. Polyomaviruses are nonenveloped icosahedral DNA viruses that are relatively small, having capsids with a diameter of about 45 nm. Their genomes consist of a single copy of a double-stranded, covalently closed, circular, supercoiled DNA and average about 5 kilobases in length (1,2).

Currently, there are twelve members of the genus *Polyomavirus* recognized by the International Committee on Taxonomy of Viruses (ICTV). The six best-characterized members of the group are BK virus (BKV), JC virus (JCV), simian virus 40 (SV40), murine polyomavirus (PyV), hamster papovavirus (HaPV), and lymphotropic papovavirus (LPV). The most intensely studied viruses in this group are PyV and SV40 (3). The other six polyomaviruses that are recognized by the ICTV include budgerigar fledgling disease virus (BFDV), bovine polyomavirus (BPyV), another murine polyomavirus [Kilham virus (KV)], baboon polyomavirus 2 (PPV-2), rabbit kidney vacuolating virus (RKV), and simian virus agent 12 (SA12). Rat polyomavirus (RPV) has been reported but is not yet recognized by the ICTV (4).

The circular genomes of all polyomaviruses are superficially arranged in a similar manner, and three general regions can be identified based on function. These are referred to as the regulatory (noncoding) region, the early coding region, and the late coding region. The regulatory region separates the coding regions and contains nucleotide sequences that are necessary for the initiation of viral DNA replication and that govern the balance between early and late transcription. The direction of early and late transcription is divergent, with opposite DNA strands used during these processes (1,2).

Multiple strains have been identified for some but not all members of the genus *Polyomavirus*. For example, SV40 strains 776 (5), Baylor (6–8), and VA45-54 (5,7–9) have been widely used in laboratory studies. Each SV40 strain was derived from an independent isolate of the virus, and DNA sequence polymorphisms in genetically stable regions of the viral genome outside of the viral regulatory region were used to distinguish strains (7,8). The avian polyomavirus referred to as BFDV by the ICTV is not a single entity; three distinct viruses can be distinguished based on DNA sequence analysis and clinical studies, and these three viruses are designated BFDV 1–3 (10). In contrast, studies of polyomavirus KV have been based on a single virus isolate. It is likely that multiple strains would be identified for more polyomaviruses, such as KV, if additional isolates of a virus were characterized in detail. It should be emphasized that strain designations based on DNA polymorphisms do not necessarily suggest serological variations. The complete DNA sequences of many polyomaviruses have been deposited at GenBank. The GenBank genomic accession numbers for viruses that are considered reference strains by the ICTV are shown in table 1.

3. POLYOMAVIRUS BIOLOGY IN NATURAL HOSTS

Mammalian polyomavirus infections are typically subclinical and persistent in their natural hosts (11). Persistence occurs in multiple organs and for indefinite periods (12–22). Unlike their mammalian

Table 1. Reference strains of polyomaviruses[a]

VIRUS	DESIGNATION	GENBANK GENOMIC ACCESSION NO.
BK virus	BKV (strain Dunlop)	[J02038]
Bovine polyomavirus	BPyV	[D00755]
Hamster papovavirus	HaPV	[X02449]
JC virus	JCV (strain Mad-1)	[J02226]
Kilham virus	KV	[M55904]
Lymphotropic papovavirus	LPV (strain K38)	[K02562]
Murine polyomavirus	PyV (strain A2)	[J02288]
Simian virus 40	SV40 (strain 776)	[J02400]

Notes: [a] Designation by the International Committee on Taxonomy of Viruses.

Table 2. Examples of cytolytic diseases associated with polyomavirus infections in natural hosts

VIRUS	HOST	CYTOLYTIC DISEASE	REFERENCE NO.
BFDV-1	Birds	Multisystemic disease	24
BKV	Humans	Acute hemorrhagic cystitis	25–28
		Acute respiratory tract disease	15
		Meningoencephalitis	29
		Urethral stenosis in renal allograft recipient	30
		Ulceration in renal allograft recipient	30
		Tubulointerstitial nephritis	31
JCV	Humans	PML[a]	32–34
SV40	Monkeys	PML	35–38
		Interstitial pneumonia	39
		Renal tubular necrosis	39
		Neurological lesions	40,41

Notes: [a] Progressive multifocal leukoencephalopathy

counterparts, avian polyomaviruses are sometimes agents of clinically apparent diseases. The original BFDV isolate, now referred to as BFDV-1, was recovered from fledgling budgerigars and causes a multisystemic infection with high mortality rates. BFDV-2 was isolated from chickens (but has not yet been associated with disease in chickens), whereas BFDV-3 was isolated from a macaw exhibiting symptoms similar to those observed with BFDV-1 infection (10). Thus, two of the three known avian polyomaviruses are recognized as agents of acute lethal disease, unlike the usually benign infections caused by mammalian polyomaviruses.

Polyomaviruses replicate their DNA and form mature progeny virus particles in the nucleus of host cells. They are able to complete their life cycle in both permissive and semipermissive cells. Efficient viral growth occurs in permissive cells, resulting in the production of relatively large amounts of progeny virions and cell death. Viral growth in semipermissive cells is less efficient, resulting in low yields of progeny virions. The time required to complete the viral growth cycle varies according to cell type, as well as other factors. In contrast, infection of nonpermissive cells does not result in the production of infectious progeny. Such infections are believed to lead to abortive infection, with subsequent rapid loss of the viral genome from the infected cell, and only rarely, random integration of portions of the viral DNA into the host chromosomal DNA (23).

Under rare circumstances, usually associated with some form of immunosuppression of the host, polyomaviruses may elicit one of two distinctly different types of disease: (i) cytolytic disease and (ii) tumor formation. Examples of cytolytic diseases associated with polyomavirus infections in their natural hosts are listed (table 2).

Based on studies of BKV, JCV, PyV, and SV40, the ability of these viruses to occasionally induce tumors in their natural hosts is thought to be a consequence of a combination of rare circumstances, as tumor induction is not a part of the normal virus life cycle. These circumstances would probably include a cellular block that depresses new virus production, but not early gene expression, thereby preventing cell killing, coupled with a failure of the host immune response to kill or inhibit the outgrowth of virus-altered cells. To date, polyomaviruses BKV, HaPV, JCV, SV40 and PyV have been detected in association with tumors arising in their natural hosts (table 3). Tumor formation in natural hosts by polyomaviruses has been experimentally confirmed for the rodent viruses HaPV and PyV. Those studies have been performed using large inocula of virus, as large viral inputs can overcome some normal immune responses. Neonatal animals with immature immune systems are usually required in order to obtain a high frequency of tumor induction. The significance of detection of BKV and JCV DNA in certain human tumors is harder to assess, as it is not possible to

Table 3. Examples of polyomaviruses detected in association with tumors in their natural hosts

VIRUS	TUMOR TYPE	REFERENCE NO.
BKV	Cerebral tumors	42–45
	Osteosarcomas	43
HaPV	Skin epithelioma	46
JCV	Astrocytomas	47,48
SV40	Astrocytoma	49
PyV	Epithelial tumors	50,51
	Osteosarcomas	50,51
	Fibrosarcomas	50,51
	Other tumors	50,51

conduct direct experiments to establish whether those viruses have oncogenic potential in humans.

It has not been established whether polyomavirus lytic disease in the natural host precedes tumor formation or whether tumor formation may occur concomitantly with cytolytic disease in the same animal. Precedence for the latter possibility has been presented for JCV, as JCV-induced PML is sometimes observed in patients with cerebral tumors, such as multiple gliomas and astrocytomas (discussed in Ref. 47). If tumor formation is a protracted event, it is conceivable that any virus-induced lytic disease is resolved before cancer becomes apparent. Alternatively, it is possible that the two disease spectra are unrelated, with the development of each dependent on distinct defects of the immune system. The type of tissue affected in lytic disease may reflect the site at which a particular polyomavirus establishes a latent or persistent infection. For example, the kidney is thought to be the target organ for both BKV and SV40, and each virus has been found in association with kidney disease (table 2).

4. POLYOMAVIRUS-INDUCED TUMORS IN FOREIGN HOSTS

A brief overview will be given of current concepts in polyomavirus tumor biology. Polyomaviruses are classified as small DNA tumor viruses, despite the fact that tumor formation is not a primary function of the viruses. The observation that polyomaviruses have oncogenic potential arose from studies which showed that selected viruses could induce tumors when inoculated into animal species that were unrelated to their natural host (3). BKV, for example, is oncogenic in hamsters (reviewed in Ref. 52,53), mice (54), and rats (55,56). The cross-species oncogenic capability of JCV has been demonstrated using Syrian hamsters (53,57–60), adult owl monkeys (61), and squirrel monkeys (62). PyV is oncogenic in newborn hamsters (63) and newborn rats (64). SV40 was shown to be oncogenic in newborn hamsters (9,65,66), in the African rodent *Rattus natalensis* (67), and in certain inbred strains of mice (68).

The rodent model systems have certain limitations that make them unable to accurately reflect the possible pathogenesis of virus-induced tumors in humans. As nonrodent polyomaviruses do not replicate well, if at all, in rodent tissues, the tissue tropisms, patterns of viral dissemination within the host, and replication-related events cannot be duplicated. Rather, large quantities of virus are injected into the animals, sometimes directly into sites such as the brain. Normal hosts would not be subjected to viral infection by such traumatic routes nor would they encounter as large an infective dose of polyomaviruses as a single exposure event. Moreover, it is not clear if all polyomaviruses have the capability to induce tumors in experimental animals. For example, inoculation of KV into experimental animals did not result in tumor formation (69–71), and LPV failed to induce tumor formation in newborn hamsters (72).

The ability of intact polyomaviruses, or the appropriate genomic DNA fragments encoding for viral oncoproteins, to transform cells has been explored using tissue culture systems (3). In general, when whole virus particles are used, nonpermissive cells are most readily transformed by polyomaviruses because progeny virus is not produced and the cells are not killed. If early gene expression can occur, polyomavirus tumor antigens will be produced and expression of those proteins past a cell-specific threshold level will lead to an alteration of the normal cell cycle, eventually resulting in cell transformation.

Using cultured cells, it has been shown that BFDV-1 transforms nonpermissive hamster embryo cells (73). BKV has the capacity to transform permissive or semipermissive human cells, nonpermissive or semipermissive monkey cells, and nonpermissive hamster, mouse, rabbit, and rat cells (reviewed in Ref. 52,53). HaPV DNA transforms primary rat embryo fibroblasts (74), which are nonpermissive for virus replication. JCV reportedly can transform cultured human and hamster cells, but inefficiently (reviewed in Ref. 53,75). LPV has the capacity to transform nonpermissive hamster embryo cells (reviewed in Ref. 52). PyV transforms hamster and rat cells that are either semipermissive or nonpermissive for the virus (76). Finally, SV40 has the capability to transform human cells, as well as nonpermissive rodent cells (76; reviewed in Ref. 77). These studies showed that *in vitro* there is a broad host range with respect to polyomavirus-mediated transformation potential, as transformation is not limited to certain specific cell types.

The oncogenic potential of polyomavirus subgenomic fragments encoding for viral tumor proteins has also been demonstrated using transgenic animal models. Transgenic mice expressing the large tumor antigen (T-ag) of JCV (78), LPV (79,80), or SV40 (79,80) develop tumors, and similar abnormalities are detected when the different viral oncoproteins are expressed in common cells. Thus, using these transgenic mouse models, it has been suggested that the biochemical events leading to cellular transformation by the T-ags of JCV, LPV, and SV40 are similar. Unlike what is observed in tissue culture models, the tumors that are formed tend to be cell-type specific, with the affected cell type being largely dependent on the promoter used to express the T-ags (reviewed in Ref. 81).

5. POLYOMAVIRUS DNA IN TUMOR TISSUE — EPISOMAL VS. INTEGRATED

A common conclusion from the early rodent studies was that the viral genomic DNA associated with polyomavirus-induced tumors was always integrated into host-cell chromosomal DNA. However, more recent data show that, although viral DNA is commonly found to be integrated in rodent cells, episomal viral DNA may persist in some cells. The impression about viral integration status may have stemmed, in part, from the notion that infection of nonpermissive cells resulted in rapid clearance of the viral DNA and that viral DNA was retained in those cells if it became integrated. Sporadic reports of rescue of infectious virus from tumors were explained by one of several viral excision mechanisms that might have accounted for the release of the viral DNA from its site of integration (76); the possibility of long-term persistence of episomal copies of viral DNA was not considered. These ideas were popularized subsequent to experiments involving analysis of SV40 infections of nonpermissive mouse cells. Murine cells stably transformed by SV40 contained viral DNA that was integrated into the chromosomal DNA (82), and at least the early region of the viral genome was retained as transformation required expression of SV40 T-ag (83). Integration of the SV40 DNA was nonspecific with respect to sites on both the viral and host DNAs and usually resulted in loss of portions of the viral genome; however, the complete viral genome could be present, as integration of tandemly multimerized viral DNA sometimes occurred (84–91). It is known that polyomaviruses do not encode for integrases and that the viral DNA becomes integrated into host chromosomes at very low frequencies and at random sites. Thus, integration into the host chromosome is an incidental event and not a normal part of the polyomavirus life cycle.

The hypothesis that polyomavirus DNA is rapidly lost from nonpermissive and semipermissive environments appears to be an over-simplification. Induction of tumors by SV40 in rodents depends on the age and genetic background of the host at the time of virus exposure, with a cytotoxic T lymphocyte response mediating tumor rejection (92). However, it has been reported that tumor induction in mice by SV40 was controlled by long-term viral persistence as well as by the host's immune response (93). *H-2*^{d} haplotype mice, which exhibit a low cytotoxic T lymphocyte response to SV40 tumor-specific transplantation antigen, formed tumors in response to virus exposure, whereas *H-2*^{k} high-responder mice did not. In more recent studies, long-term persistence of SV40 in mice was confirmed; marker gene expression driven from the SV40 promoter was detected more than one year after infection of mice (94). Long-term persistence of JCV in hamsters has also been demonstrated, and viral gene expression has been shown to be tissue specific. Hamsters sacrificed 6 months postinfection with JCV contained virus in brain, kidney, and bladder tissue, although JCV-specific gene expression was confined primarily to brain tissue (95).

It is noteworthy that episomal polyomavirus DNA has been detected in various types of tumors and transformed tissue-culture cells. Although studies have not carefully compared the frequency of episomal versus integrated polyomavirus DNAs in tumors, numerous observations indicate that episomal copies commonly persist. Defective episomal BKV DNAs together with integrated BKV DNA was found in a human papillary urothelial bladder carcinoma; the episomal BKV DNA had apparently acquired a cellular DNA sequence, forming chimeric episomal DNA molecules that were larger than unit-length BKV DNA (96). Human fetal brain cells transformed by BKV contained episomal viral DNA (97). Infectious BKV was rescued from human brain tumors, from African Kaposi's sarcoma tissues, and from three human tumor cell lines (glioblastoma, neuroblastoma, and osteogenic sarcoma) (44), most likely reflecting the presence of nonintegrated viral DNA. Tumors induced in hamsters by BKV were found to contain predominantly integrated viral DNA, but some tumors also contained free viral sequences (98). Infectious JCV was molecularly cloned from tumor cells derived from a JCV-induced owl monkey astrocytoma that had been passaged twice in culture (99). HaPV particles (containing wild-type DNA) were associated with hair follicle epitheliomas in the Z3/BlnA Syrian hamster breed (46), whereas infection of HaP Syrian hamsters led to the formation of lymphomas containing defective episomal HaPV DNA (100). With one exception (101), the PyV genome is predominantly present in an unintegrated state in PyV-induced tumors arising in immunocompetent mice (discussed in Ref. 51); integrated PyV DNA appears to occur with high frequency, however, when athymic (nude) mice are tested (51,102–104). In contrast, in transformed rat embryo cells, the PyV genome was observed to be present in both integrated and unintegrated forms (105–107). Infectious SV40 has been isolated from tumors induced in hamsters by inoculation of SV40. Examples of tumor-derived isolates are SV40 strain A2895 (108), and D-128-15 (Lednicky, unpublished observations). Infectious viral DNA was demonstrated in SV40-transformed hamster, rat, mouse, and monkey cells (109,110). Human fibroblasts transformed by plasmids containing the early region of SV40 DNA (encoding for the viral oncoproteins) reportedly retained the plasmids in an episomal manner; eventually the plasmids were lost and the cells underwent senescence (111). Human glioblastoma cells persistently infected with SV40 acquired a more transformed phenotype yet carried nondefective episomal

Table 4. Reports of polyomavirus DNA in tumor and/or normal tissue from humans

VIRUS	TISSUE TYPE	VIRAL DNA[a]		REFERENCE NO.
		TUMOR TISSUE	NORMAL TISSUE	
BKV	Brain	+	+	43,117
	Brain	+	–	42,44,45
	Brain	–	+	13,118
	Kidney carcinoma	+		119
	Osteosarcoma	+	+	43
	Osteosarcoma	+		44
	Papillary urothelial bladder carcinoma	+		96
	Peripheral blood cells		+	117,120,121
	B lymphocytes		+	117
	T lymphocytes		+	117
JCV	Brain	+		47,48
	Brain		+	13,118,122
	Cardiac muscle		+	123
	Kidney		+	13,124,125
	Liver		+	16,123
	Lung		+	16,123
	Peripheral blood cells		+	120,121,126–128
	Spleen		+	16,123
	B lymphocytes		+	129,130
SV40	Brain	+		113,114,131–136
	Malignant melanoma	+		115
	Mesothelioma	+		137–142
	Mesothelioma	+	+	143
	Osteosarcoma	+		144–146
	Pituitary	+	+	147
	Peripheral blood cells		+	117
	B lymphocytes		+	117
	T lymphocytes		+	117
	Thyroid carcinoma	+	–	148

Notes: [a] + = Detection of viral DNA; – = failure to detect viral DNA.

viral DNA (112). SV40 was cloned from a meningioma (113), isolated and cloned from a choroid plexus tumor (114), and isolated from a metastatic melanoma (115).

It is not clear at this time if episomal polyomaviral genomes alone are sufficient to maintain the transformed state or if an integrated viral DNA copy is required. The fact that polyomavirus DNA is sometimes found in an episomal manner in tumor tissue is reminiscent of the situation with human papillomavirus strain 16 (HPV-16). In HPV-16-associated cancers, the viral DNA is either integrated or episomal, whereas in HPV-18-associated cancers, the viral DNA is almost always integrated. HPV-16 predominates in squamous cell carcinomas, whereas HPV-18 predominates in adenocarcinomas and in small-cell undifferentiated carcinomas of the cervix (reviewed in Ref. 116). It is apparent, therefore, that strain differences among the papillomaviruses account for different types of diseases, as well as influence whether the viral DNA remains episomal or integrated in a tumor; the same situation may hold true for polyomaviruses.

6. ASSOCIATION AND SIGNIFICANCE OF POLYOMAVIRUS DNA WITH HUMAN TUMORS

There are accumulating reports of the association of polyomaviruses BKV, JCV, or SV40 with certain human tumors (table 4). It is striking that all three viruses have been associated with brain tumors, emphasizing a basic neurotropic character of primate polyomaviruses. Investigators studying the possible association of polyomaviruses with human tumors are faced with two difficult tasks: (i) detection of viral DNA and/or viral gene products, and (ii) evaluation of the significance of viral presence in tumor tissue.

Older studies relied primarily on DNA hybridization methods, such as Southern blot analysis or DNA reassociation kinetics, and on immunological methods, such as indirect immunofluorescence studies using antibody against polyomavirus tumor antigens, to search for papovavirus DNA sequences or virus-encoded proteins in tumor tissue. More recent studies have depended primarily on sensitive polymerase chain reaction (PCR) methodology to detect viral sequences in human samples. The exquisite sensitivity of PCR requires confirmation of the specificity of presumed virus-specific amplified DNA products by sequence analysis or by Southern blot analysis (149,150). This is necessary as the PCR primers may amplify homologous DNA sequences from closely related viruses or from nonpolyomavirus DNAs under certain conditions, and this could lead to misleading results (151). Some studies have reported finding viral sequences present in both tumor and normal

tissues or in normal tissue alone (table 4). It should be noted that not all PCR-based studies have detected polyomavirus DNAs in association with tumors, complicating the interpretation of these findings (152–154). The facts that BKV and JCV are known to commonly establish persistent infections in people and to be excreted in the urine by individuals who are asymptomatic complicate the evaluation of their potential role in the development of human tumors. These observations of the presence of polyomavirus sequences in normal tissues other than kidney, believed to be the target organ for BKV, JCV, and SV40, raise the theoretical possibility that there may be types of polyomavirus disease in humans that are currently unrecognized (155).

Numerous studies have validated the detection of viral sequences in human tumor tissues (3), establishing that polyomaviral DNAs were present (table 4). The question then becomes one of evaluating the biological significance of that presence. Rodent HaPV and PyV have been verified experimentally to possess oncogenic capability in their natural hosts (discussed above). Therefore, it is tempting to generalize that BKV and JCV are human tumor viruses as well and were important factors in the induction of the tumors in which they were detected. However, proof that they played significant etiological roles is lacking, and no conclusions can be drawn at this time about their status as human cancer viruses. The potential role of SV40 in human tumors is controversial at present, due to a widely held view that SV40 is unable to infect humans. However, it is evident from reports in the literature that SV40 does have the capacity to both infect and replicate efficiently in certain types of human cells (discussed in Refs. 3,7,114), including human fetal glial cells (156), in a variety of human fetal tissues and newborn human kidney cells (157), and in human fetal brain cultures and various human cell lines (158). The ability of human cells to support the replication of SV40 DNA has been demonstrated in vitro using human cell extracts (159–161), establishing that human proteins have the intrinsic ability to interact with SV40 T-ag to replicate SV40 DNA. It is worth noting that SV40 does not infect and replicate efficiently in all types of simian cells, so it is not unexpected that some human cells are nonpermissive. The potent ability of SV40 to induce tumors in foreign species, together with its ability to infect human cells, strengthens the hypothesis that SV40 might exhibit oncogenic capability in humans (3).

However, when the same virus is detected in normal tissues as in tumors, it must be considered that the virus is present in the neoplastic cells merely as a harmless "passenger". It should not be considered unusual to find polyomavirus DNA in normal tissue, as these agents establish long-term persistent infections. Accepted human oncogenic viruses are commonly detected in normal tissue; examples include human papillomaviruses, which are found in normal and precancerous cervical tissue (162; reviewed in Refs. 116,163), and hepatitis B virus, which can be found replicating in nontumorous liver tissue (164).

With most viruses, the mere detection of virus presence does not indicate the development of clinical disease; similarly, the presence of an oncovirus in tumor tissue cannot be assumed to be proof of causality. What parameters, then, can be used to establish whether a given polyomavirus is the causative agent for a human tumor in which the virus was detected? This is an important consideration in that there has not yet been a perfect correlation of any polyomavirus with a given tumor. One approach to establish causality is to demonstrate the presence of virus-specific tumor antigens in the tumor cells. Based on studies in rodents, the T-ag oncoprotein of BKV, JCV, and SV40 must be continually expressed to maintain the transformed phenotype. One way of detecting viral protein expression is to use indirect immunofluorescence with the primary antibody directed against a particular T-ag, an approach exemplified in the study by Martini *et al.* (117), who used monoclonal antibody Pab101 to detect SV40 T-ag in various human tumor cell lines. Such studies are highly indicative of the presence and expression of a viral T-ag in tumor cells, but are not definitive for virus identification purposes because the majority of available antibodies are cross-reactive with the T-ags of multiple polyomaviruses. Furthermore, lessons learned from studies of BPyV indicate that bovine serum used for tissue culture may be contaminated by this virus (165–167); it is possible that a bovine virus was present in the serum used to culture tumor cells and that BPyV-contaminated cells would react with the primary antibody used to detect T-ag. It was not clear if the cell lines studied by Martini *et al.* (117) were true positives, as the cells were obtained commercially and may have been contaminated prior to distribution. Contamination with bovine viruses is not an issue, however, when tumor samples are not exposed to bovine serum. In addition, there are monoclonal antibodies available which are reportedly specific for a given viral protein, such as pAb419 for SV40 T-ag and antibody BK-T.1 for BKV T-ag (168). The pAb419 reagent was used to demonstrate SV40 T-ag in mesotheliomas (139).

Immunohistochemical assays can be performed using antibodies directed against a particular T-ag, as reported by Bergsagel *et al.* (132). However, many antibodies that are useful in other types of assays fail to react by immunohistochemistry on paraffin-embedded tissues. Again, the limitation of such studies for virus identification purposes is the type of primary antibody used. For example, the polyclonal antibody used in the study by Bergsagel *et al.* (132) may have been cross-reactive with T-ags other than that of SV40. Antibody tests that employ cross-reactive reagents could be coupled with additional assays, such as reverse transcriptase PCR, to demonstrate expression of a specific T-ag RNA in a tumor; this approach was used by Martini *et al.* (117).

A more sophisticated approach is to demonstrate the presence of a functionally active viral T-ag in the tumor tissue. For example, a particular T-ag could be detected and confirmed, using two independent tests, and then be shown to be functional, using a test that measures a known activity of that T-ag. SV40 T-ag in mesotheliomas was shown to interact with tumor suppressor protein p53 (139) and to be able to bind the Rb family of tumor suppressor proteins (140). Unfortunately, these types of functional assays require relatively large amounts of fresh, unfixed

tumor sample. Paraffin-embedded samples, needle biopsy material, or small amounts of tumor tissue are not adequate to be tested using these procedures.

An additional level of complexity is that viruses may act as cocarcinogens, i.e., cancer development may be influenced by viral infection but require other cofactors as well. This possibility is exemplified by reports of association of SV40 with human mesotheliomas (137–143). There is a significant correlation between asbestos exposure and the development of mesotheliomas; it is possible that SV40 accelerates the development of mesotheliomas in asbestos-exposed individuals or that both asbestos and SV40 together are required to induce mesothelioma development in some persons.

7. PERSPECTIVE

Polyomaviruses are small DNA viruses that are dependent upon host cellular factors for replication, requiring the involvement of a single viral product, the T-ag. These viruses target terminally differentiated cells, which are usually in a quiescent state. Because the factors required for DNA replication are not actively expressed in quiescent cells, polyomaviruses induce the synthesis of necessary factors by stimulating entry into the cell cycle. This is mediated at least in part by the binding of viral T-ags to tumor suppressor proteins Rb and p53, leading to cellular S-phase. The side effect of this replication function is that the viruses have an intrinsic ability to transform cells under certain conditions, especially in cells resistant to a permissive viral infection.

The mere association of polyomavirus DNA with cancer tissue is not proof of causality. The main difficulties in establishing a polyomavirus as an etiologically significant factor in human cancer causation are similar to those that were encountered in studies of cancer induction by HPV (reviewed in Ref. 3,116). These difficulties are: (i) The infecting viruses cause ubiquitous infections, yet only a small percentage of infected individuals develop cancer. (ii) The time periods elapsing between primary infections and cancer development may be prolonged. (iii) Other agents (such as chemicals) are frequently suspected to be causally related to the same tumors.

To date, the number of human tumors associated with polyomaviruses is small, yet infections by BKV and JCV are ubiquitous and evidence is mounting that infections by SV40 do occur (3). There is no doubt these agents can cause cancer in experimental animals. To provide convincing evidence of an etiologic role in human tumors, functional studies will need to be carried out to analyze viral expression in the implicated tumors. Useful approaches would examine properties including the status of viral DNA in tumor cells, the percentage of virus-infected tumor cells, the expression of viral oncoproteins in tumor cells and their interaction with cellular tumor suppressor proteins, the possible strain variation of tumor-associated viruses, and possible synergism due to coinfection of tumor cells by two known polyomaviruses. If polyomaviruses are contributing to the development of human neoplasia, it will be informative to determine the unique genetic, physiological, or immunological nature of those individuals who are prone to virus-mediated tumor induction.

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