**Oncogenic Viruses**

# PRINCIPLES OF TUMOR VIROLOGY

Viral infections play a causal role in at least 10% of all new cancer diagnoses worldwide. A vast majority of cases (>85%) occur in developing countries, where poor sanitation, high rates of cocarcinogenic factors such as HIV/AIDS, and lack of access to vaccines and cancer screening all contribute to increased rates of virally induced cancers. Even in developed countries, where effective countermeasures are widely available, cancers attributable to viral infection account for at least 4% of new cases.

Viruses that are known to be carcinogenic in humans come from six distinct viral families with a range of physical characteristics (Table 7.1). All known human cancer viruses are capable of establishing durable, long- term infections and cause cancer only in a minority of persistently infected individuals. The low penetrance of cancer induction is consistent with the idea that a virus capable of establishing a durable productive infection would not benefit from inducing a disease that kills the host.3 The slow course of cancer induction (typically over a course of many years after the initial infection) suggests that viral infection alone is rarely sufficient to cause human malignancy and that virally induced cancers arise only after additional oncogenic “hits” have had time to accumulate stochastically.

In broad terms, viruses can cause cancer through either (or both) of two broad mechanisms: direct or indirect. Direct mechanisms, in which the virus-infected cell ultimately becomes malignant, are typically driven by the effects of viral oncogene expression or through direct genotoxic effects of viral gene products. In most established examples of direct viral oncogenesis, the cancerous cell remains “addicted” to viral oncogene expression for ongoing growth and viability.

A common feature of DNA viruses that depend on host cell DNA polymerases for replication (e.g., papillomaviruses, and polyomaviruses) is the expression of viral gene products that promote progression into the cell cycle. A typical mechanism of direct oncogenic effects is through the inactivation of tumor suppressor proteins, such as the “guardian of the genome,” p53, and retinoblastoma protein (pRB). This effectively primes the cell to express the host machinery necessary for replicating the viral DNA. The study of tumor viruses has been instrumental in uncovering the existence and function of key tumor suppressor proteins, as well as key cellular protooncogenes, such as Src and Myc.

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| **TABLE 7.1**  **Oncogenic Viruses** | | | | | | | |
| **Virus** | **Taxon** | **Viral Genome** | **Virion** | **Infection Rate** | **Site of Persistence** | **Diseases in Normal Hosts** | **Diseases in Immunocom Hosts** |
| High-risk human papillomavirus types (e.g., HPV16) | *Alphapapillomavirus* | 8 kb circular dsDNA | Nonenveloped | >70% | Ano-genital mucosa, oral mucosa | Carcinomas of the cervix, penis, anus, vagina, vulva, tonsils, base of tongue, bladder | Increased in same diseas |
| Hepatitis B virus | *Hepadnaviridae* | 3 kb ss/dsDNA | Enveloped | 2%–8% | Hepatocytes | Cirrhosis, hepatocellular carcinoma | Same diseas increased inc with AIDS |
| Hepatitis C virus | *Flaviviridae* | 10 kb  +RNA | Enveloped | <3% | Hepatocytes | Cirrhosis, hepatocellular carcinoma, | Same diseas increased inc with AIDS |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  | splenic marginal zone lymphoma |  |
| Epstein-Barr virus (HHV-4) | *Gammaherpesvirinae* | 170 kb linear DNA | Enveloped | 90% | B cells, pharyngeal mucosa | Mononucleosis, Burkitt lymphoma, other non- Hodgkin lymphoma, nasopharyngeal carcinoma | Increased in same diseas lymphoprolif disease, oth lymphomas, leukoplakia, leiomyosarco |
| Kaposi sarcoma herpesvirus (HHV-8) | *Gammaherpesvirinae* | 170 kb linear DNA | Enveloped | 2%–60% | Oral mucosa, endothelium, B cells | Kaposi sarcoma, multicentric Castleman disease | Increased Ka sarcoma, mu Castleman d incidence, pr effusion lymp |
| Merkel cell polyomavirus | *Alphapolyomavirus* | 5 kb circular dsDNA | Nonenveloped | 75% | Skin (lymphocytes?) | Merkel cell carcinoma | Increased M carcinoma in |
| Human T cell leukemia virus | *Deltaretrovirus* | 9 kb  +RNA (RT) | Enveloped | 0.01%–  6% | T and B cells | Adult T cell leukemia- lymphoma, tropical spastic paraparesis, myelopathy, uveitis, dermatitis | Unknown |
| BK  polyomavirus | *Betapolyomavirus* | 5 kb circular dsDNA | Nonenveloped | 90% | Bladder epithelium | None established | Cystisits, ne encephalopa |

Ranges for infection rates imply major variations in prevalence among populations in different world regions. “Associated cancers” indicates the annual number of new cases clearly attributable to viral infection. An estimate for the worldwide incidence of Merkel cell carcinoma is not currently available; an estimate of the annual new cases in the United States alone is given instead.

kb, kilobases; ds, double-stranded; ss, single-stranded; HHV, human herpesvirus; RT, reverse-transcriptase.

In principle, viruses can cause cancer via a direct “hit-and-run” mechanism. In this scenario, viral gene products may preserve cellular viability and promote cell growth in the face of otherwise proapoptotic genetic damage during the early phases of tumor development. This allows the precancerous cell to accumulate enough additional genetic hits to eventually allow for cell growth and survival independent of viral oncogene expression. This, in turn, allows for stochastic loss of viral nucleic acids from the nascent tumor, perhaps giving a cellular growth advantage due to the loss of “foreign” viral antigens that might otherwise serve as targets for immune- mediated clearance of the nascent tumor. A hit-and-run mechanism has been documented in bovine papillomavirus 4–induced gastrointestinal cancers in cattle. Hit-and-run effects have more recently been reported in a multimammate mouse model for papillomavirus-induced squamous cell carcinoma (SCC). The effect has also been suggested for human cancers associated with hepatitis B and C viruses (HBV and HCV, respectively), Epstein-Barr virus (EBV), and human papillomaviruses (HPVs), but conclusive documentation of hit-and-run in humans remains elusive.

In indirect oncogenic mechanisms, the cells that give rise to the malignant tumor have never been infected by the virus. Instead, the viral infection is thought to lead to cancer by attracting inflammatory immune responses that, in turn, lead to accelerated cycles of tissue damage and regeneration of noninfected cells. In some instances, virally infected cells may secrete paracrine signals that drive the proliferation of uninfected cells. At a theoretical level, it may be difficult to distinguish between indirect carcinogenesis and hit-and-run direct carcinogenesis because, in both cases, the metastatic tumor may not contain viral nucleic acids.

A variety of hunting approaches have been used to uncover etiologic roles for viruses in human cancer. The first clues that high-risk HPVs, EBV, Kaposi sarcoma–associated herpesvirus (KSHV), and Merkel cell polyomavirus (MCV) might be carcinogenic were based on the detection of virions, viral DNA, or viral RNA in the tumors these viruses cause. A common feature of known virally induced cancers is that they are more prevalent in immunosuppressed individuals, such as individuals suffering from HIV/AIDS or patients on immunosuppressive therapy after organ transplantation. This is thought to reflect the lack of immunologic control over the cancer-causing virus. Studies focused on AIDS-associated cancers provided the first evidence for the

carcinogenic potential of KSHV and MCV. A theoretical limitation of this approach is that some virally induced cancers may not occur at dramatically elevated rates in all types of immunosuppressed subjects, particularly if the virus causes only a fraction of cases (e.g., HPV-induced head and neck cancers). Fortunately, the unbiased analysis of nucleic acid sequences found in tumors has become substantially more tractable as deep-sequencing methods have continued to fall in price. In coming years, it should be increasingly possible to search for viral sequences without making the starting assumption that all virally induced tumors are associated with immunosuppression.

One limitation of tumor-sequencing approaches is that they might miss undiscovered divergent viral species within viral families known to have extensive sequence diversity and could miss viral families that have not yet been discovered. Tumor-sequencing approaches might also miss viruses that cause cancer by hit-and-run or indirect mechanisms. It is conceivable that this caveat could be addressed by focusing on sequencing early precancerous lesions thought to ultimately give rise to metastatic cancer or by focusing sequencing efforts on tumors affecting immunosuppressed patients, who may exert less immunologic pressure for the virus to “run” from the nascent tumor.

An additional successful approach to hunting cancer viruses involves showing that individuals who are infected with a particular virus have an increased long-term risk of developing particular forms of cancer. This approach was successful for identifying and validating the carcinogenic roles of high-risk HPV types, HBV, HCV, KSHV, and human T-lymphotropic virus 1 (HTLV-1). Although viruses that are extremely prevalent, such as EBV, MCV, and BK polyomavirus (BKV), are not amenable to this approach per se, it may still be possible to draw connections between cancer risk and either unusually high serum antibody titers against viral antigens or unusually high viral load. Relatively high serologic titers reflect either comparatively poor control of the viral infection in at-risk individuals or expression of viral antigens in tumors or tumor precursor cells.

The finding that a virus causes cancer can suggest possible paths to clinical intervention. These can include the development of vaccines or antiviral agents that prevent, attenuate, or eradicate the viral infection and thereby prevent cancer; the development of methods for early detection or diagnosis of cancer based on assays for viral nucleic acids or gene products; or the development of drugs or immunotherapeutic interventions that treat cancer by targeting viral gene products. Unfortunately, establishing the carcinogenicity of a given viral species is an arduous process that must inevitably integrate multiple lines of evidence. The demonstration that the virus can transform cells in culture and/or cause cancer in animal models provides circumstantial evidence of the oncogenic potential of a virus. All known human cancer viruses except KSHV meet this criterion. However, it is important to recognize that viruses can theoretically coevolve to be noncarcinogenic in their native host (e.g., humans) and cause cancer only in the dysregulated environment of a nonnative host animal. This caveat may apply to human adenoviruses.

Finding that viral DNA is clonally integrated in a primary tumor and its metastatic lesions helps address the caveat that the virus might merely be a hitchhiker that finds the tumor cell a conducive environment in which to replicate (as opposed to playing a causal carcinogenic role). This caveat is also addressed by the observation that, in most instances, viruses found in tumors have lost the ability to exit viral latency and are functionally unable to produce new progeny virions. An unfortunate consequence of this is that vaccines or antiviral agents that target virion proteins (e.g., vaccines against high-risk HPVs or HBV) or gene products expressed late in the viral life cycle (e.g., herpesvirus thymidine kinase, which is the target of drugs such as ganciclovir) are rarely effective for treating existing virally induced tumors.

Demonstrating that a vaccine or antiviral agent targeting the virus either prevents or treats human cancer is by far the strongest form of evidence that a given virus causes human cancer. This type of proof has fully validated the causal role of HBV in human liver cancer. Compelling clinical trial data also show that antiherpesvirus therapeutics can prevent KSHV- or EBV-associated lymphoproliferative disorders and that vaccination against HPV can prevent the development of precancerous lesions on the uterine cervix.

# PAPILLOMAVIRUSES

## History

The viral family *Papillomaviridae* is named for the benign skin warts (papillomas) that some members of the family cause. In the early 1930s, Richard Edwin Shope and colleagues demonstrated viral transmission of papillomas in a rabbit model system. Using this system, Peyton Rous and others showed that cottontail rabbit

papillomavirus-induced lesions can progress to malignant skin cancer. This was the first demonstration of a cancer-causing virus in mammals, building on Rous’ prior work demonstrating a virus capable of causing cancer in chickens (the Rous sarcoma retrovirus).

The idea that cancer of the uterine cervix might be linked to sexual behavior was first proposed in the mid-19th century by Dominico Rigoni-Stern, who observed that nuns rarely contracted cervical cancer, whereas prostitutes suffered from cervical cancer more often than the general populace. Another major milestone in cervical cancer research was Georgios Papanikolaou’s development of the so-called Pap smear for early cytologic diagnosis of precancerous cervical lesions.15 This form of screening, which allows for surgical intervention to remove precancerous lesions, has saved many millions of lives in developed countries, where public health campaigns have made testing widely available.

Although observations in the early 1980s suggested the possibility of a hit-and-run carcinogenic role for herpes simplex viruses in cervical cancer, this hypothesis was abandoned in light of studies led by Harald zur Hausen. Low-stringency hybridization approaches revealed the presence of two previously unknown papillomavirus types, HPV16 and HPV18, in various cervical cancer cell lines, including the famous HeLa cell line. There is now overwhelming evidence that a group of more than a dozen sexually transmitted HPV types, including HPV16 and HPV18, play a causal role in essentially all cases of cervical cancer. HPVs associated with a high risk of cancer also cause about half of all penile cancers, 88% of anal cancers, 43% of vulvar cancers, 70% of vaginal cancers, and an increasing fraction of head and neck cancers. In 2008, zur Hausen was awarded the Nobel Prize for his groundbreaking work establishing the link between HPVs and human cancer.

## Tissue Tropism and Gene Functions

Although papillomaviruses can achieve infectious entry into a wide variety of cell types in vitro and in vivo, the late phase of the viral life cycle, during which the viral genome undergoes vegetative replication and the L1 and L2 capsid proteins are expressed, is strictly dependent on host cell factors found only in differentiating keratinocytes near the surface of the skin or mucosa. Interestingly, a majority of HPV-induced cancers appear to arise primarily at zones of transition between stratified squamous epithelia and the single-layer (columnar) epithelia of the endocervix, the inner surface of the anus, and tonsillar crypts. It is thought that the mixed phenotypic milieu in cells at squamocolumnar transition zones may cause dysregulation of the normal coupling of the HPV life cycle to keratinocyte differentiation.

There are more than 300 known HPV types (https://pave.niaid.nih.gov/). In general, each papillomavirus type is a functionally distinct serotype, meaning that serum antibodies that neutralize one HPV type do not robustly neutralize other HPV types. Various HPV types preferentially infect different skin or mucosal surfaces and tend to establish either transient infections that may be cleared over the course of months or stable infections where virions are chronically shed from the infected skin surface for the lifetime of the host. HPV infections may or may not be associated with the formation of visible warts or other lesions. High-risk HPV types, with clearly established causal links to human cancer, are preferentially tropic for the anogenital mucosa and the oral mucosa, are usually transmitted by sexual contact, rarely cause visible warts, and usually establish only transient infections in a great majority of exposed individuals. The lifetime risk of sexual exposure to a high-risk HPV type has been estimated to be >70%. Individuals who fail to clear their infection with a high-risk HPV type and remain persistently infected are at much greater risk of developing cancer. Polymerase chain reaction–based screening for the presence of high-risk HPV types thus serves as a useful adjunct to, or even a replacement for, the traditional Pap test.

A consequence of the strict tissue-differentiation specificity of the papillomavirus life cycle is that HPVs do not replicate in standard monolayer cell cultures. Papillomaviruses also seem to be highly species restricted, and there are no known examples of an HPV type capable of infecting animals. Thus, the investigation of key details of papillomavirus biology has relied on modern recombinant DNA and molecular biologic analyses.

Papillomavirus genomes are roughly 8 kb, double-stranded, closed-circular DNA molecules (essentially reminiscent of a plasmid). During the normal viral life cycle, the genome does not adopt a linear form, does not integrate into the host cell chromosome, and remains as an extrachromosomal episome or minichromosome. All the viral protein-coding sequences are arranged on one strand of the genome. The expression of various proteins is regulated by differential transcription and polyadenylation, as well as effects at the level of RNA splicing, export from the nucleus, and translation. In addition to the late half of the viral genome, which encodes the L1 and L2 capsid proteins, HPVs encode six key early region genes: E1, E2, E4, E5, E6, and E7.

The master transcriptional regulator E2 serves as a transcriptional repressor, and loss of E2 expression

(typically through integration of the viral episome into the host cell DNA) results in the upregulation of early gene expression. The most extensively studied early region proteins are the E6 and E7 oncogenes of HPV16 and HPV18. The E6 protein of high-risk HPV types triggers the destruction of p53 by recruiting a host cell ubiquitin– protein ligase, E6AP. Another important oncogenic function of E6 is the activation of cellular telomerase. A wide variety of additional high-risk E6 activities that do not involve p53 have been identified.

Most E7 proteins, including those of many low-risk HPV types, contain a conserved LXCXE motif that mediates interaction with pRB and the related “pocket” proteins p107 and p130. Interestingly, the LXCXE motif is present in a wide variety of other oncogenes, most notably the T antigens of polyomaviruses and the E1A oncogenes of adenoviruses. The interaction of E7 with pRB disrupts the formation of a complex between pRB and E2F transcription factors, thereby blocking the ability of pRB to trigger cell cycle arrest. The E7 proteins of high- risk HPVs can also contribute to chromosomal missegregation and aneuploidy, which may in turn contribute to malignant progression. Like E6, E7 interacts with a wide variety of additional cellular targets, the spectrum of which seems to vary with different HPV types.

Some papillomavirus types express an E5 oncogene, which functions as an agonist for cell surface growth factor receptors such as platelet-derived growth factor β (PDGF-β) and epidermal growth factor (EGF) receptor. Although E5 plays a critical role in tumor development in some animal papillomaviruses, and likely plays a role in viral immune evasion in precancerous lesions, E5 expression is uncommon in cervical tumors, and it remains uncertain whether the protein plays a key role in human cancer.

## Human Papillomavirus Vaccines

Two preventive vaccines against cancer-causing HPVs, trade named Gardasil 9 (Merck) and Cervarix (GSK), are currently marketed worldwide for the prevention of cervical cancer. Both vaccines contain recombinant L1 capsid proteins based on HPV16 and HPV18 that are assembled in vitro into virus-like particles (VLPs). Together, HPV16 and HPV18 cause about 70% of all cases of cervical cancer worldwide. Gardasil 9 also includes VLPs based on an additional five HPV types associated with cervical cancer as well as HPV types 6 and 11, which rarely cause cervical cancer but together cause about 90% of all genital warts. The VLPs contained in the vaccines are highly immunogenic in humans, eliciting high-titer serum antibody responses against L1 that are capable of neutralizing the infectivity of the cognate HPV types represented in the vaccine. The vaccines effectively prevent the development of cervical intraepithelial neoplasias that are known to give rise to cervical cancer. Antibody responses elicited by the vaccines are highly durable and are expected to confer lifelong immunity against new infection with the HPV types represented in the vaccine. The vaccines elicit lower titer cross-neutralizing responses against a subset of cancer-causing HPV types that are closely related to the types represented in the vaccine.

Multiple studies conducted worldwide have found no increased risk of adverse events among HPV vaccine recipients. Nevertheless, uptake of HPV vaccines has been slow in some countries, in part due to sociopolitical controversy and false public perception of risk. A 2016 survey indicates that about 60% of U.S. adolescents ages 13 to 17 have received at least one dose of the vaccine. Despite the limited uptake, Markowitz and colleagues found a 61% decrease in the prevalence of HPV16 and HPV18 in the United States from 2009 to 2012. Decreases in the prevalence of HPV16/18 and in the incidence of genital warts have been even more pronounced in countries, such as Australia, that had better HPV vaccine uptake rates.

Because L1 is not expressed in latently infected keratinocyte stem cells residing on the epithelial basement membrane, current HPV vaccines are unlikely to eradicate existing infections. Like keratinocyte stem cells, cervical cancers and precursor lesions rarely or never express L1. Thus, the existing L1-based vaccines seem unlikely to serve as therapeutic agents for treating cervical cancer.

Two types of next-generation HPV vaccines are currently in human clinical trials. One newer vaccine approach targets the papillomavirus minor capsid protein L2. An N-terminal portion of L2 appears to represent a highly conserved “Achilles’ heel” epitope that is required for key steps of the infectious entry process. Antibodies against this portion of L2 can neutralize a broad range of different human and animal papillomavirus types, and vaccines targeting L2 could thus offer protection against all HPVs that cause cervical cancer, all low-risk HPV types that may cause abnormal Pap smear results, and the full range of HPV types that cause skin warts. Another category of vaccines seeks to elicit T-cell-mediated immune responses against the E6 and E7 oncoproteins. Because expression of E6 and E7 is critical for tumor development and survival, such vaccines could provide a therapeutic intervention for eradication of precancerous lesions and treatment of cervical cancer.

The ongoing dependence of cervical carcinomas on E6 and E7 expression makes this type of cancer an

appealing target for newer immunotherapeutic approaches.

## Oropharyngeal Cancer

It is well established that tobacco products and alcohol cause head and neck cancer. In the late 1990s, Maura Gillison and colleagues noted a surprising number of new cases of tonsillar cancer in nonsmokers. Many of the tumors found in nonsmokers were found to have wild-type p53 genes, raising the possibility that the tumor might be dependent on a p53-suppressing viral oncogene (as seen in cervical cancer). Gillison and colleagues went on to show that nearly half of all tonsillar cancers contain HPV DNA, most commonly HPV16. Interestingly, HPV- positive oropharyngeal cancers tend to be less lethal than tobacco-associated HPV-negative tumors. This finding has important implications for treatment of HPV-positive head and neck cancers.

Although the incidence of tobacco-associated head and neck cancer has been declining in recent decades due to decreased tobacco use, recent studies suggest an ongoing increase in the incidence of HPV-associated cancers of the tonsils and the base of the tongue. By 2025, the number of new HPV-induced head and neck cancer cases in the United States is expected to roughly equal the number of new cervical cancer cases. Based in part on these observations, the Centers for Disease Control and Prevention recommends that boys, in addition to girls, should be vaccinated against high-risk HPVs.

As with cervical carcinoma, HPV-positive head and neck cancers have attracted significant attention as possible targets for immunotherapeutic approaches.

## Nonmelanoma Skin Cancer

Epidermodysplasia verruciformis is a rare immunodeficiency that is characterized by the appearance of numerous wart-like lesions across wide areas of skin. The lesions typically contain papillomaviruses from genus *Beta*, such as HPV5 or HPV8. Epidermodysplasia verruciformis patients frequently develop SCC in sun-exposed skin areas (suggesting that ultraviolet light exposure is a cofactor). It is also well established that other immunosuppressed individuals, such as organ transplant recipients and HIV-infected individuals, are at increased risk of developing SCC. Although the E6 and E7 proteins of betapapillomaviruses appear to exert a different spectrum of effects than the E6 and E7 proteins of HPV types associated with cervical cancer, betapapillomavirus oncogenes can transform cells in vitro. Although these circumstantial lines of evidence suggest that infectious agents, such as betapapillomaviruses, might play a causal role in SCC, deep sequencing studies have observed few or no viral sequences in SCC tumors. Although the results argue against durable direct oncogenic effects of any known viral species in SCC, an animal model system using bovine papillomavirus type 4 strongly suggests that papillomaviruses can cause cancer by hit-and-run mechanisms. More recent studies in a multimammate mouse papillomavirus model system have documented a hit-and-run mechanism for development of SCC.5 It seems likely that a similar mechanism is at play in some fraction of human SCC cases.

## Bladder Cancer

Although HPVs are generally thought of as exhibiting a strict tropism for stratified squamous epithelial tissues, some animal papillomaviruses, such as bovine papillomavirus type 2, are known to infect the stratified epithelium of the bladder. In cattle exposed to cocarcinogenic agents found in bracken fern, bovine papillomavirus type 2 causes bladder carcinoma. In humans, a subset of bladder carcinomas exhibit an HPV-induced histologic pattern called koilocytosis and such lesions are often positive for HPV16 or HPV18. More recent cancer genomics studies have confirmed that a small fraction of bladder carcinomas carry HPV sequences integrated into the tumor genome and that such tumors are, like HPV-induced cervical or head and neck cancers, less likely to carry mutations in key tumor suppressor genes, such as p53. The results strongly suggest that HPVs are involved in at least a small fraction of bladder cancer cases.

# POLYOMAVIRUSES

## History

In the early 1950s, Ludwik Gross showed that a filterable infectious agent could cause salivary gland cancer in laboratory mice. Later work by Bernice Eddy and Sarah Stewart showed that the murine polyoma (Greek for

“many tumors”) virus caused many different types of cancer in experimentally infected mice. The discovery that murine polyomavirus could be grown in cell culture helped rekindle research interest in the question of whether viruses might cause human cancer.

Like papillomaviruses, polyomaviruses have a nonenveloped capsid assembled from 72 pentamers of a single major capsid protein (VP1). Both viral families also carry circular double-stranded DNA genomes. These physical similarities initially led to the classification of both groups into a single family, *Papovaviridae*. When sequencing studies ultimately revealed that polyomaviruses have a unique genome organization (with early and late genes being arranged on opposing strands of the genome) and essentially no nucleotide sequence similarity to papillomaviruses, the two groups of viruses were divided into separate families.

In the early 1960s, Bernice Eddy, Maurice Hilleman, and Benjamin Sweet reported the discovery of simian vacuolating virus 40 (SV40), a previously unknown polyomavirus that was found as a contaminant in vaccines against poliovirus. SV40 was derived from the rhesus monkey kidney cells used to amplify poliovirus in culture.44 SV40 rapidly became an important model polyomavirus, and studies of its major and minor tumor antigens (large T [LT] and small t [sT], respectively) have played an important role in understanding various aspects of carcinogenesis. Despite significant alarm about the possible risk SV40 might pose to exposed individuals, a comprehensive, decades-long series of studies have failed to uncover compelling evidence that SV40 exposure is causally associated with human cancer.

The first two naturally human-tropic polyomaviruses, BK virus (BKV) and JC virus (JCV), were first reported in back-to-back publications in 1971. BKV and JCV (later designated HPyV1 and HPyV2, respectively) are known to cause kidney disease and a lethal brain disease called progressive multifocal leukoencephalopathy, respectively, in immunosuppressed individuals. Like their close relative SV40, BKV and JCV can cause various forms of cancer in experimentally exposed animals.

## BK Polyomavirus

Integrated BKV sequences have been conclusively documented in two tumors from a panel of 412 muscle- invasive bladder carcinomas. The virus has also been found in several dozen cases of urinary carcinomas affecting transplant recipients. Several recent epidemiologic studies have shown that kidney transplant patients with a clinically documented failure to control BKV replication are at significantly increased risk of developing invasive bladder carcinoma. Taken together, these data strongly suggest that BKV plays a persistent directly carcinogenic role in a small percentage of bladder cancer.

Although BKV LT expression can frequently be observed in the inflammatory precursor lesions that are thought to give rise to prostate cancer, there is no evidence for persistence of BKV DNA in malignant prostate tumors. It remains possible that BKV plays a hit-and-run role in an additional fraction of cancers of the urinary epithelium.

## Merkel Cell Polyomavirus

In 2008, Yuan Chang and Patrick Moore reported their lab’s discovery of the fifth known human polyomavirus species, which they named MCV (later designated HPyV5) based on its presence in Merkel cell carcinoma (MCC). The discovery used an RNA deep-sequencing approach called digital transcriptome subtraction. Using classic Southern blotting, the initial report demonstrated the clonal integration of MCV in an MCC tumor and its distant metastases. Many other laboratories worldwide have independently confirmed the presence of MCV DNA in about 80% of MCC tumors.

MCC is a rare but rapidly lethal form of cancer that typically presents as a fast-growing violaceous lesion on sun-exposed skin surfaces (Fig. 7.1). The risk of MCC is dramatically higher in HIV/AIDS patients and organ transplant patients, offering an initial clue that MCC might be a virally induced cancer. Although MCC tumors express neuroendocrine markers associated with sensory Merkel cells of the epidermis, one report has suggested that some MCC tumors also express B-cell markers, including rearranged antibody loci. Currently, there is no clear evidence for involvement of MCV in other tumors with neuroendocrine features.

In 2012, the International Agency for Research on Cancer (IARC) classified MCV as a class 2A carcinogen (probably carcinogenic to humans). It should be noted that IARC evaluations rely heavily on animal carcinogenicity studies, and the 2A designation was assigned prior to a more recent report showing that MCV- positive MCC lines are tumorigenic in a mouse model system.

A great majority of healthy adults have serum antibodies specific for the MCV major capsid protein VP1. A

majority also shed MCV virions from apparently healthy skin surfaces, and there is a strong correlation between individual subjects’ serologic titer against VP1 and the amount of MCV DNA they shed. Interestingly, MCC patients tend to have exceptionally strong serologic titers against VP1. MCC tumors do not express detectable amounts of VP1, so this is unlikely to reflect direct exposure to the tumor and instead likely represents a history of a high MCV load in MCC patients. A study of archived serum samples shows that unusually high serologic titers against MCV VP1 often precede the development of MCC by many years.

Like other polyomavirus LT proteins (and the E7 proteins of high-risk HPVs), an N-terminal portion of the MCV LT contains an LXCXE motif that mediates inactivation of pRB function. In contrast to SV40 and BKV LT, which carry a p53-inactivation domain that overlaps the C-terminal helicase domain, MCV LT does not appear to inactivate p53 function. Instead, the MCV LT helicase domain activates DNA damage responses and induces cell cycle arrest in cultured cell lines. This may explain why the LT genes found in MCC tumors essentially always carry mutations that truncate LT upstream of the helicase domain. siRNA experiments indicate that most (although possibly not all) MCC tumors are “addicted” to the expression of MCV T antigens. Rapid progress has recently been made toward understanding the signaling pathways that MCV gene expression disrupts, and many of these insights are currently being tested in MCC clinical trials. These fast-moving translationally oriented efforts have recently been reviewed.

Patients with higher levels of MCV DNA in their tumors, stronger T-antigen expression, and tumors that have been infiltrated by CD8+ T cells appear to have better prognoses. This is consistent with the idea that cell- mediated immunity can help clear MCC tumors that express MCV antigens. Immunomodulatory therapies, such as “checkpoint inhibitor” approaches, have shown clinical responses in 30% to 50% of patients with advanced MCC, and follow-up studies are in progress.



**Figure 7.1** Merkel cell carcinoma (MCC). The left panel shows an MCC tumor on the calf. The right panel shows an MCC tumor on the finger. (Photographs provided with permission by Dr. Paul Nghiem, University of Washington, [www.merkelcell.org](http://www.merkelcell.org).)

## Other Human Polyomaviruses

In recent years, the number of known human polyomaviruses has expanded dramatically. Of the 12 currently known HPyV species, only BKV and MCV have been clearly linked to human cancer. One new HPyV, trichodysplasia spinulosa polyomavirus has been found in association with abnormal spiny growths on the facial skin of a small number of immunocompromised individuals. Recent reports have suggested that HPyV6 and HPyV7 may play a causal role in pruritic skin rashes with a distinctive “peacock tail” histology in patients with various forms of immunosuppression. One report indicates that HPyV7 DNA and T-antigen expression can often be detected in thymic tumors. This observation has not yet been addressed by other laboratories, and it remains unclear whether HPyV7 plays a causal role in thymic tumors.

# EPSTEIN-BARR VIRUS

## History

In 1958, Denis Burkitt described an unusual B-cell–derived tumor that most frequently arises in the jawbones of children in equatorial Africa. The first description of this tumor dates back to 1896, when Albert Cook, a missionary doctor in Uganda, reported a child with a large jaw mass. After hearing Burkitt give a 1961 lecture entitled “The Commonest Children’s Cancer in Tropical Africa—A Hitherto Unrecognized Syndrome,” Michael Epstein became interested in the idea that an insect vector-borne infection might account for the high incidence of Burkitt lymphoma in tropical Africa. Epstein, together with then PhD candidate Yvonne Barr, began examining tumor samples sent to them by Dr. Burkitt. Electron micrographs of lymphoid cells that grew out of the tumors in culture revealed viral particles with a morphology strikingly similar to herpes simplex viruses. It was soon shown that Epstein-Barr herpesvirus (EBV; later designated human herpesvirus 4) can immortalize primary human B cells, giving rise to long-term proliferating lymphoblastoid cell lines in vitro. The virus is responsible for acute infectious mononucleosis, a generally benign lymphoproliferative disease.

Although Epstein’s initial conjecture was that tropically endemic Burkitt lymphoma depends on a geographically restricted infectious agent, it was quickly established that over 90% of adults worldwide are asymptomatically infected with EBV and that sporadic forms of Burkitt lymphoma are EBV-positive in <50% of tumors. These imperfect correlations initially suggested that EBV is merely a passenger in cancer rather than a driver of oncogenesis. There is now a consensus that EBV is a bona fide tumor virus; EBV was declared a class I carcinogen by the IARC and the World Health Organization in the late 1990s. EBV is estimated to be responsible for about 200,000 cancers worldwide. However, the mechanisms of EBV tumorigenesis remain under investigation and appear diverse. In endemic Burkitt lymphoma, the malaria parasite *Plasmodium falciparum* is likely a key geographically restricted cocarcinogen. In areas where children suffer repeated malaria infections, it appears that the parasite weakens T-cell–mediated immunity and promotes B-cell proliferation, leading to aberrant expression of activation- induced cytidine deaminase. These effects of recurring malaria infection increase the likelihood of a successful oncogenic c-myc translocation, a central driver of Burkitt tumor pathogenesis.

## Epstein-Barr Virus Life Cycle

In most individuals, initial EBV infection occurs asymptomatically in early childhood. The infection is typically transmitted through the saliva of EBV-seropositive individuals who periodically replicate the virus in the oropharyngeal epithelium. The EBV envelope glycoprotein gp350 binds with high affinity to the B-cell–specific CD21 complement receptor, which mediates virus attachment to B lymphocytes followed by virus entry and establishment of long-term nonproductive infection. B lymphocytes are abundant in the tonsillar crypts and mediate systemic dissemination of EBV infection. Individuals who escape infection during childhood and instead first become infected during adolescence or adulthood often develop infectious mononucleosis, a syndrome associated with fever, lymphadenopathy, pharyngitis, and fatigue. Besides having EBV-infected B cells and EBV DNA in the circulation, patients with acute infectious mononucleosis typically have T-cell lymphocytosis, which usually declines in weeks. Interestingly, late-infected individuals who experience mononucleosis and high EBV viral load are at increased risk of developing EBV-positive Hodgkin lymphoma.

After primary infection, EBV persists in the host by establishing latency in a small number of resting B cells and undergoing periodic replication, mostly in the oropharyngeal epithelium. Viral latency is defined as a condition in which the virus expresses one or few gene products but can, under some conditions, “reawaken” to express a broader range of viral gene products. Latently infected cells are highly resistant to immune clearance.

Although the pattern of EBV latent gene expression can be heterogeneous, a simplified classification recognizes three forms of EBV latency. In latency I, EBV nuclear antigen-1 (EBNA1), which is required for the stable maintenance of the circularized viral DNA minichromosome, is the only viral protein expressed. EBV- derived microRNAs (miRs) may also be expressed. At the other end of the spectrum, latency III is characterized by the expression of EBNA1–6, several latent membrane proteins (LMP1, LMP2A, and LMP2B), two noncoding RNAs (EBER1 and 2), the BCL-2 homolog BHRF1, RK-BARF0, and multiple miRs. Although the initial discovery of EBV involved the visualization of virions, indicating that the virus had exited latency and entered the productive lytic phase of the life cycle, viral gene expression in EBV-induced cancers generally follows one of the three latent patterns. The oncogenic activities of various EBV gene products have been reviewed.

Primary and chronic EBV infection is controlled by innate immunity and by adaptive immunity directed at various latency proteins. EBV, like other herpesviruses, expresses a variety of proteins that interfere with cell- mediated immune responses, which, together with its ability to establish latency, could explain why the virus is not eradicated after primary infection. Intriguingly, results from mouse model systems suggest that the chronic immunostimulatory effects of persistent gammaherpesvirus emergence (or abortive emergence) from latency in healthy hosts can nonspecifically boost immunity to other infections.

## Lymphomas

Nearly all cases of endemic Burkitt lymphoma are EBV-positive. By contrast, EBV is present in only about 20% of sporadic cases of Burkitt lymphoma that occur in immunocompetent individuals outside of malaria-prone regions. Individuals infected with HIV are known to have a 60- to 200-fold increased risk of Burkitt and other non-Hodgkin lymphomas, and about half of HIV-associated lymphomas contain EBV.

A hallmark of all types of Burkitt lymphomas is deregulation of the cellular Myc protooncogene. A classic mutation involves chromosomal translocation of the Myc gene to the antibody heavy chain locus. Burkitt lymphoma tumors that lack detectable EBV DNA tend to carry multiple additional mutations in host cell genes. This is consistent with a tumorigenic role of EBV and has raised the possibility of a hit-and-run scenario in which an originally EBV-positive precursor cell ultimately accumulated mutations that rendered it independent of viral genes. EBV-negative derivatives of EBV-positive Burkitt cell lines provide evidence that EBV does not play an obligatory role in the growth of EBV-positive Burkitt tumor cells.

In addition to Burkitt lymphoma, EBV is associated, to a varying extent, with a histologically diverse range of other lymphoid cancers, including posttransplant lymphoproliferative disease (PTLD), mixed cellularity and lymphocyte-depletion subsets of Hodgkin and other lymphomas and natural killer (NK)/T-cell lymphoma. PTLD develops in the setting of T-cell immunosuppression following bone marrow or solid organ transplantation, particularly when the patients are EBV-naive prior to transplant. PTLD is typically of B-cell origin, and the EBV- infected B cells are frequently polyclonal. Oligoclonal or monoclonal forms are also observed, particularly in cases that arise long after transplantation. Oncogenes and tumor suppressor gene alterations typical of other lymphomas are often observed in such cases. A fulminant and often fatal EBV+ mononucleosis-like lymphoproliferative disease is observed in patients with X-linked lymphoproliferative disease (XLP), an immunodeficiency caused by mutations in the *SH2D1A* gene, which confers a selective vulnerability to EBV infection. EBV is also associated with rare lymphoproliferative diseases of non–B-cell origin, including NK cell leukemia, and nasal-type NK/T cell (angiocentric) lymphoma.

HIV infection confers an increased risk of developing aggressive B-cell lymphomas and Hodgkin lymphomas. Lymphoma is a classic AIDS-defining condition, and HIV infection status should be tested at presentation. Patients with AIDS also develop primary central nervous system (CNS) lymphoma and infrequently plasmablastic lymphoma; both are almost always EBV-infected. Current diagnostic tools for early diagnosis and treatment have rendered AIDS-associated CNS lymphoma a potentially curable disease. Overall, long-term survival for HIV-associated lymphoma has increased from <20% prior to antiretroviral therapy to >80% for most lymphoma types, with outcomes similar to the general population for large B-cell lymphoma, Burkitt lymphoma, and Hodgkin lymphoma.

## Carcinomas

In southern China, nasopharyngeal carcinoma (NPC) affects 25 out of 100,000 people, accounting for 18% of all cancers in China. Most other world regions have a 25- to 100-fold lower rate of NPC. EBV is present in nearly all cases of NPC, both in endemic and nonendemic regions. Although there is support for the idea that dietary intake of salted fish and other preserved foods is a factor in endemic NPC, it remains possible that genetic traits or as yet unidentified environmental cocarcinogenic factors may play a role as well. Individuals with rising or relatively high immunoglobulin A antibody responses to EBNA1, EBV DNase, and/or capsid antigens are at increased risk of developing NPC, offering an early detection method for at-risk individuals.

EBV is also present in a small percentage (5% to 15%) of gastric adenocarcinomas and over 90% of gastric lymphoepithelioma-like carcinomas. In contrast to NPC, the prevalence of EBV-associated gastric cancer is similar in all world regions. As with NPC, elevated antibody responsiveness to EBV antigens may offer a method for identifying individuals at greater risk of gastric cancer.

## Prevention and Treatment

The reduction of immunosuppressive drugs in response to increasing EBV loads has proven effective for preventing EBV diseases in some T-cell immunosuppressed individuals. Improved control of HIV infection has resulted in decreased incidence of certain EBV-associated lymphoproliferative diseases such as primary CNS lymphoma. Ganciclovir (or related antiherpesvirus drugs) can significantly reduce EBV replication by inhibiting

viral DNA polymerase, and small studies have suggested a role for antiviral treatment of PTLD and other EBV- associated lymphoproliferative diseases, but conclusive data are currently lacking.

Most forms of EBV-associated lymphoid cancers express the B-cell marker CD20, making rituximab (an anti- CD20 mAb) a potentially effective adjunct therapy. In addition, EBV-specific T cells generated ex vivo by stimulation with EBV peptides or autologous EBV-infected B cells have shown promise as prophylaxis and therapy for PTLD, raising the possibility that they may be developed into effective treatments against EBV- positive malignancies.

Development of an EBV prophylactic vaccine has been difficult. The most recently developed vaccine targeting the EBV gp350 virion surface antigen did not provide sterilizing immunity to EBV infection, but EBV- negative vaccinees experienced a reduced rate of infectious mononucleosis but no change in the rate of EBV infection.

# KAPOSI SARCOMA HERPESVIRUS

## History and Epidemiology

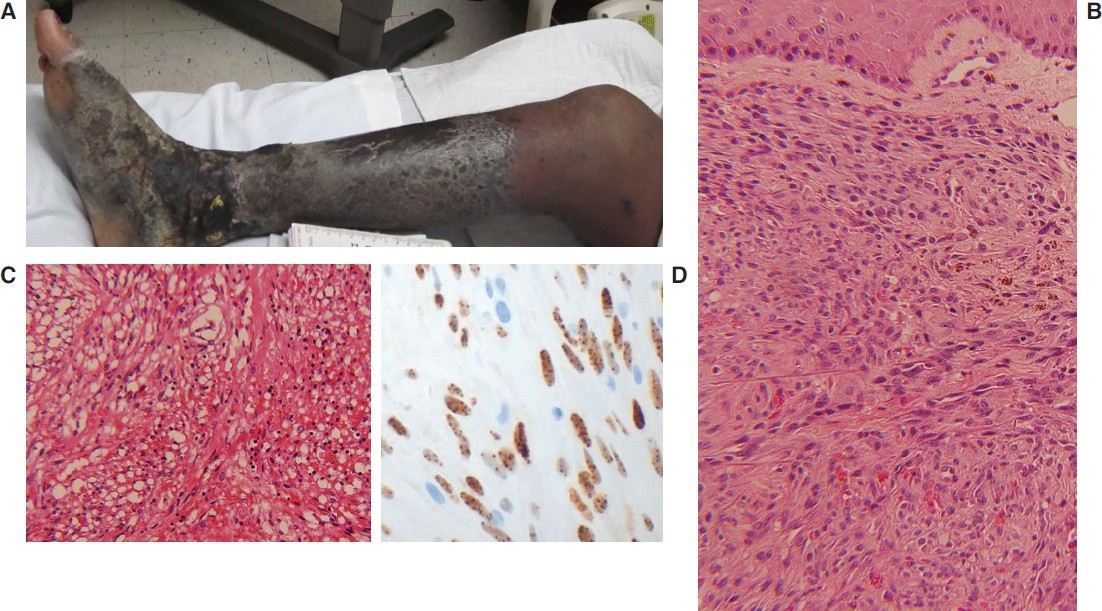
In the late 19th century, Hungarian dermatologist Moritz Kaposi described a relatively rare type of indolent pigmented skin sarcoma affecting older men. Kaposi sarcoma (KS) was later found to be more prevalent in the Mediterranean region and in eastern portions of sub-Saharan Africa. An early clue to the emergence of the HIV/AIDS pandemic in the 1980s was a dramatic increase in the incidence of highly aggressive forms of KS, particularly among gay men who were much younger than typical KS patients. The observation that KS was significantly more common in men who have sex with men compared to other HIV-infected groups, in conjunction with other epidemiologic factors, suggested that KS was more easily explained by the existence of a sexually transmitted cofactor other than HIV.

In 1994, using a subtractive DNA hybridization approach known as representational difference analysis, Moore, Chang, and colleagues discovered the presence of a previously unknown herpesvirus in KS tumors. The newly founded field of research rapidly established key lines of evidence supporting the conclusion that KSHV (later designated human herpesvirus-8) is a causal factor for all KS types, including classical KS in elderly men, AIDS-associated (epidemic) KS, transplantation-associated KS, and endemic KS in sub-Saharan Africa.

The rate of KSHV infection varies greatly in different world regions. In North America and Western Europe, KSHV seroprevalence in the general population ranges from 1% to 7%. Seroprevalence among gay men in these regions is substantially higher (25% to 60%), suggesting potential sexual transmission. Seroprevalence in sub-Saharan Africa and countries around the Mediterranean Sea is similarly high (23% to 70%). In Uganda, up to 30.6% of 8-year-old children are seropositive, suggesting transmission via nonsexual casual contact (presumably via saliva). KS rarely affects immunocompetent individuals in North America. In HIV-infected individuals, the risk of developing KS is inversely related to CD4+ cell count, and control of HIV infection with antiretroviral treatment has decreased the incidence of KS in the United States. KS remains the most common cancer in some African nations ([http://globocan.iarc.fr/Pages/Map.aspx).](http://globocan.iarc.fr/Pages/Map.aspx))

## Kaposi Sarcoma–Associated Herpesvirus in Kaposi Sarcoma

KS is a multicentric tumor, generally presenting with multiple lesions that do not reflect metastatic disease. In contrast to most other forms of cancer, where the malignant cell population is often dominant and clearly identified, KS tumors contain cells from multiple lineages (Fig. 7.2). The malignant KSHV-infected cells often have a spindle-shaped morphology and are of endothelial lineage, although endothelial cell markers are not consistently present. When infected with KSHV, primary endothelial cells undergo a process of endothelial-to- mesenchymal transformation, during which they lose endothelial cell markers and acquire expression of mesenchymal markers, explaining the complex phenotype of KSHV-infected spindle cells in KS lesions. KS tumors also contain infiltrating lymphocytes, plasma cells, monocytes, endothelial cells, and fibroblasts that are not KSHV-infected, and these additional cell types are believed to provide essential signals for the survival and growth of the tumor cells. Most KS lesions display aberrant slit-like leaky spaces replete with red cells and lined with infected and uninfected endothelial cells that rupture easily and leak red blood cells, giving KS tumors their classic dark red, brown, or purple color.



**Figure 7.2** Kaposi sarcoma. **A:** Photograph of the lower leg of an individual with severe, diffuse Kaposi sarcoma involving the lower leg. **B:** Histology of the skin. **C:** Lung shows a mixture of spindle to epithelioid cells, with slitlike vascular spaces intermixed with red blood cells and red blood cell fragments. **D:** Immunohistochemical detection of Kaposi sarcoma–associated herpesvirus latency-associated nuclear antigen in the cutaneous tumor. (Photographs provided with permission by Drs. Odey Ukpo and Ethel Cesarman.)

The latency status of KSHV in KS tumors is complex, with the expression of gene products typical of latency (e.g., latency-associated nuclear antigen [LANA]) as well as lytic-phase genes (e.g., RTA/ORF50). Some of these gene products, such as the viral interleukin (IL)-6 homolog (vIL-6) and a G-coupled protein receptor product of ORF74, trigger proliferation and secondary cytokine signaling in noninfected cells within the tumor. The tumorigenic effects of individual KSHV gene products have been reviewed. In contrast to EBV, where tumorigenesis is driven by latency-associated gene expression, it appears that KS development is often dependent on lytic phase gene expression. This may explain why ganciclovir, which targets the late-phase thymidine kinase gene, can prevent the formation of new KS lesions in HIV-positive.

There are a variety of possible explanations for the need for lytic-phase KSHV gene expression during tumor development. For example, infected spindle cells may lose the viral DNA during cell division and require reinfection for ongoing tumorigenicity. Alternatively, factors secreted by a small fraction of tumor cells that enter the lytic phase may be required for tumorigenesis. Currently, treatment of AIDS-KS starts with combination antiretroviral therapy, which can induce significant antitumor immune responses, especially in antiretroviral therapy-naive patients. Patients who do not respond to antiretroviral therapy are most frequently treated with systemic administration of pegylated liposomal doxorubicin, with paclitaxel as second-line therapy. Many patients achieve long-term control.

## Lymphoproliferative Disorders

KSHV causes two forms of B-cell proliferative disorders: multicentric Castleman disease (MCD) and primary effusion lymphoma (PEL). MCD occurs at increased frequency in AIDS patients; in this group, MCD is almost invariably KSHV-positive. MCD also occurs, if rarely, in HIV-negative individuals, and in this group, only 40% to 50% of cases are KSHV-infected. MCD is a severe systemic illness, characterized by intermittent flares of inflammatory symptoms, including fever, night sweats, and wasting attributable to acute cytokine release, particularly cellular and viral IL-6, as well as IL-10. The patients often have lymphadenopathy, splenomegaly, anemia, thrombocytopenia, hyponatremia, increased levels of C-reactive protein, low albumin, and increased KSHV viral load that is attributable to activation of lytic replication. The diagnosis of MCD is based on histologic detection of LAN-positive plasmablasts in the mantle region of affected lymph nodes that often show a vascularized core. Until recently, the median survival of patients with MCD was less than 2 years. With the

advent of new therapies, including rituximab alone or with liposomal doxorubicin plus high-dose zidovudine and valganciclovir, the prognosis is markedly improved.

PEL comprises about 4% of all HIV-associated non-Hodgkin lymphomas. Typically, PEL presents as a liquid malignancy in body cavity effusions. The tumor cells, generally clonal, are uniformly infected with KSHV and in 80% of cases are coinfected with EBV. PEL cells display rearranged Ig genes but do not express markers of mature B cells. Rather, they resemble “B1” lymphocytes, a population of B-cells that secrete broadly reactive antibodies important for innate immune defense, and which are frequently detected in mouse body cavities. Although the cell of origin of PEL has been uncertain, recent results suggest that KSHV-infected mesothelial cells give rise to PEL cell through a process of transdifferentiation. In addition to symptoms related to the presence of an effusion, PEL patients often have inflammatory symptoms attributable to elevated serum inflammatory cytokines. Treatment of PEL with chemotherapy regimens used for aggressive lymphomas in combination with antiretroviral therapy can lead to long-term remissions in <40% of patients.

# ANIMAL AND HUMAN RETROVIRUSES

The first oncogenic retroviruses were discovered by Ellerman and Bang in 1908 and by Rous in 1911, but it was many years before the significance of these findings was appreciated. One reason the field was stymied was the failure to identify RNA forms of the viral genome in infected cells. This led to the discovery of reverse transcriptase independently by Baltimore and Temin in 1970. Another major development was the finding, in 1976, of viral oncogenes derived from cellular genes, with the identification by Varmus and Bishop of the first dominant oncogene, *src*. With the discovery of IL-2 by Gallo in 1976, it became possible to culture the first human retrovirus, HTLV-1, from a form of adult T-cell leukemia/lymphoma (ATLL) that was first recognized by Takatsuki and coworkers. These advances opened the door for Montagnier and colleagues’ isolation of HIV-1 in 1983, a discovery confirmed independently by Gallo and Levy. This breakthrough led to the first licensed HIV test in 1985.

Retroviruses are positive single-strand RNA viruses that utilize transcription of their RNA genome into a DNA intermediate during virus replication. This accounts for their name, retroviruses, because this is opposite to the normal flow of eukaryotic genetic information. They infect a wide range of animal species and are distantly related to repetitive elements in the human genome, known as retrotransposons. Retroviruses are also related to hepadnaviruses, double-stranded DNA viruses, such as hepatitis B virus, which also undergo a reverse- transcription step in their replication.

Retroviruses may be classified as *endogenous* or *exogenous* depending on whether they appear in the germline of the host species. There are approximately 100,000 endogenous retroviral elements in the human genome, making up nearly 8% of the genetic information, but their potential roles in disease are unclear. Retroviruses may also be classified as *ecotropic*, *xenotropic*, or *polytropic* depending on whether they infect cells of the same animal species from which they are derived, infect cells of a different species, or both. *Amphotropic* retroviruses infect cells of the species of origin without producing disease but infect cells of other species and may produce disease.

Retroviruses that produce disease after a long incubation period are termed *lentiviruses* and include human, simian, feline, ovine, caprine, and bovine immunodeficiency viruses. Another group of retroviruses that are not clearly associated with disease are known as *spumaviruses* and include human and simian foamy viruses. HTLV- 1, which is classified in the genus Delta, is the only retrovirus known to be oncogenic in humans. A member of the retroviral genus Gamma identified in 2008, designated xenotropic murine leukemia virus-related virus (XMRV), was thought to be associated with human prostate cancer; however, subsequent studies showed XMRV to be a lab-derived artifact. A genus Beta retrovirus related to mouse mammary tumor virus has been suggested to be associated with biliary cirrhosis and human breast cancers, but there is debate about whether these findings might represent sample contamination.

Retroviruses producing tumors in animals or birds are designated transforming viruses and may be classified as acute or chronic transforming retroviruses. Acute transforming retroviruses have acquired a mutated cellular gene, termed *oncogene*, and induce cancer in an animal within a few weeks. Many dominant acting protooncogenes in humans (e.g., *ras*, *myc*, and *erbB*) were first identified as acute transforming retroviral oncogenes.

Chronic transforming retroviruses integrate almost randomly in the genome and can disrupt the regulation of nearby genes and induce cell proliferation or resistance to apoptosis. Chronic transforming retroviruses induce malignancy only after many weeks to months of infection. The use of a murine leukemia virus vector for gene therapy in children with a form of severe combined immune deficiency syndrome characterized by defective expression of the common gamma chain of the IL-2 receptor resulted in T-cell acute lymphoblastic leukemia. This was found to be the result of persistent expression of the LMO2 (LIM domain only 2) gene triggered by the nearby integration of the retroviral vector.

In addition to acute or chronic transformation mechanisms, retroviruses can transform cells through direct effects mediated by structural or nonstructural viral proteins. Transforming genes of HTLV-1 are nonstructural viral proteins that activate host cell signaling pathways. Because the oncogenic effects of HTLV-1 transforming genes generally take many years to cause cancer, the virus does not fit the precise definition of having either an acute or a chronic oncogenic mechanism.

HIV-1 infection is also associated with a variety of malignancies but only through indirect effects of suppressing immunity to oncogenic virus infections, such as gammaherpesviruses, high-risk human papillomaviruses, or polyomaviruses.

## Human T-Cell Leukemia Virus Epidemiology

Four species of human T-cell leukemia virus have been identified. HTLV-1 was identified in 1980 as the first human retrovirus associated with cancer, and it is the focus of the remainder of this section. HTLV-2 was discovered in 1982 and shares roughly 64% genomic sequence similarity with HTLV-1. HTLV-3 and HTLV-4 were sporadically isolated from individuals who had contact with monkeys. HTLV-2, HTLV-3, and HTLV-4 are not known to be associated with disease in humans.

HTLV-1 is present in 15 to 20 million individuals worldwide, most commonly in the Caribbean Islands; South America; southern Japan; and parts of Australia, Melanesia, Africa, and Iran. In the United States, Canada, and Europe, 0.01% to 0.03% of blood donors are infected with HTLV-1. It is most commonly found in individuals who emigrated from endemic regions or among African Americans. HTLV-1 is transmitted sexually, by contaminated cell-associated blood products, or by breastfeeding. Only 2% to 5% of HTLV-1–infected individuals develop disease.

## Human T-Cell Leukemia Virus Molecular Biology

HTLV-1, like other retroviruses, encodes Gag, Protease, Pol, and Envelope proteins. Gag proteins compose the inner nucleocapsid core of the virus. The Pol proteins include the reverse transcriptase and integrase. The reverse transcriptase copies the single-stranded viral RNA into double-stranded DNA, and it is inhibited by several nucleoside analogs, but not by nonnucleoside reverse transcriptase inhibitors approved for HIV-1. The integrase is responsible for inserting the linear double-stranded DNA product of reverse transcription into the host chromosomal DNA. At least one integrase inhibitor, raltegravir, now approved for HIV-1, is active against HTLV-1. Integration occurs throughout the human genome, but there is preference for integration into transcriptionally active genomic regions. The viral protease proteolytically processes Gag, Protease, and Pol precursor proteins to the mature individual proteins, but it is not affected by inhibitors of HIV-1 protease. The HTLV-1 envelope is cleaved by a cellular furin protease into a transmembrane and a surface component. Envelope mediates cellular attachment and fusion by binding cell-surface receptors, which are thought to include glucose transporter 1, neuropilin 1, and various heparan sulfate proteoglycans.

The viral genome also encodes regulatory proteins, including Tax and HTLV-1 bZIP factor (HBZ). Tax is a transcriptional transactivator protein that functions as a coactivator of members of the cAMP response element- binding protein/activating transcription factor (CREB/ATF) family, nuclear factor kappa B (NF-κB), and serum response factor (SRF) pathways. Tax activation of the cAMP response element-binding protein/activating transcription factor pathway is responsible for upregulation of the viral promoter. Tax induction of NF-κB promotes cell proliferation and resistance to apoptosis. Tax also binds and activates cyclin-dependent kinases and inhibits cell cycle checkpoint proteins. Tax is important for tumor initiation, whereas HBZ may be important in tumor maintenance. Once ATLL develops, Tax expression is repressed as an immune-evasion mechanism. Genetic alterations in ATLL cells replace Tax activity through constitutive activation of the T-cell receptor and NF-κB pathways.

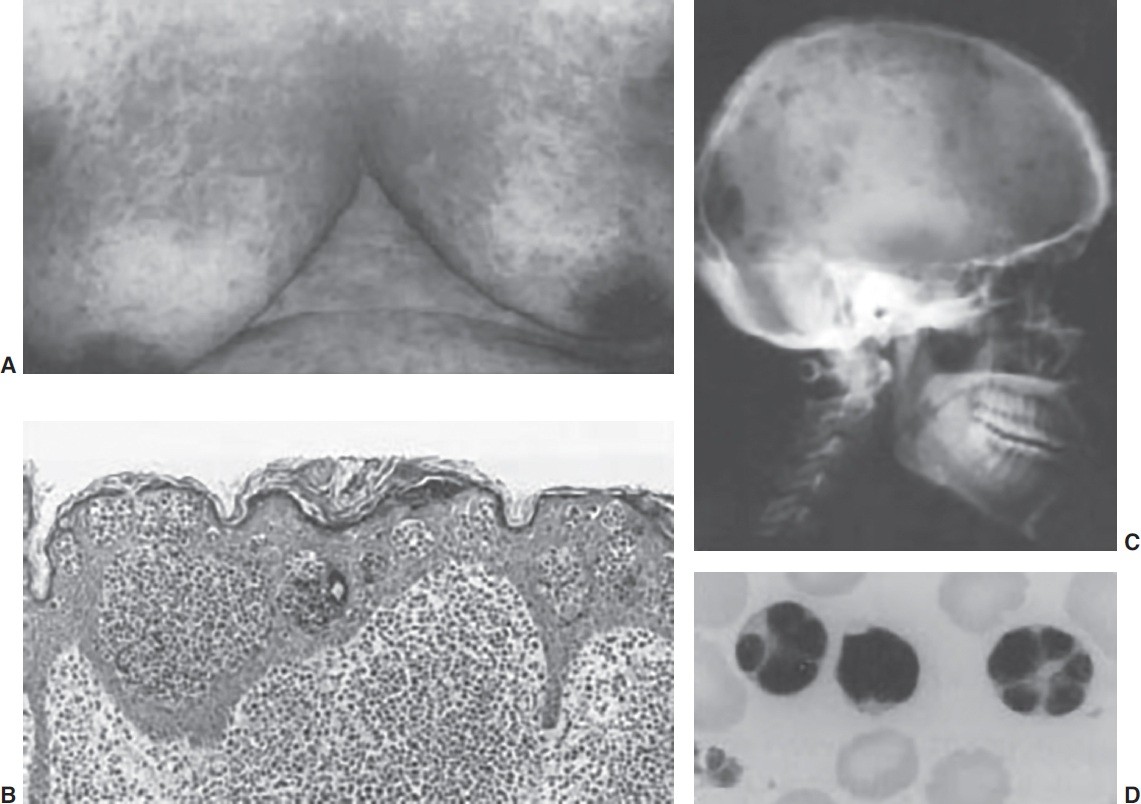
HTLV-1 preferentially immortalizes CD4+ T lymphocytes and induces tumors in mice. Tax also promotes leukemia-initiating activity of ATLL cells in mouse models. In immunodeficient mice reconstituted with human hematopoietic cells, HTLV-1 causes CD4+ lymphomas.

## Clinical Characteristics and Treatment of Human T-lymphotropic Virus 1– Associated Malignancies

The diagnosis of HTLV-1 infection is based on serologic assays. HTLV-1 is associated with various inflammatory disorders, including uveitis, polymyositis, pneumonitis, Sjögren syndrome, and myelopathy. Infected patients are susceptible to certain infectious disorders (e.g., staphylococcal dermatitis) and opportunistic infections such as pneumocystis pneumonia, disseminated cryptococcosis, strongyloidiasis, or toxoplasmosis. Vaccines have not been developed for HTLV infections.

T-lymphocyte proliferative disorders develop in 1% to 5% of infected individuals and are generally CD2+, CD3+, CD4+, CD5+, CD25+, CD29+, CD45RO+, CD52+, HLA-DR+, T-cell receptor αβ+, and variably CD30+ and lack CD7, CD8, and CD26 expression. The virus is clonally integrated in the malignant cells. Complex karyotypes are often found and cytogenetic analysis is rarely useful. The histologic features of lymph nodes in ATLL may be indistinguishable from those of other peripheral T-cell lymphomas. Circulating tumor “flower cells” are helpful in the diagnosis (Fig. 7.3).

ATLL is categorized in four subtypes: (1) Smoldering ATLL is defined as having 5% or more abnormal T lymphocytes and lactate dehydrogenase (LDH) levels up to 1.5× the upper limit of normal, with normal lymphocyte count and calcium levels, and no lymph node or visceral disease other than skin or pulmonary disease. (2) Chronic ATLL is characterized by lymphocytosis; LDH up to twice the upper limit of normal; no hypercalcemia; and no CNS, bone, pleural, peritoneal, or gastrointestinal involvement, although the lymph nodes, liver, spleen, skin, or lungs may be involved. The mean survival of these forms of ATLL is 2 to 5 years. No intervention in these subtypes of ATLL has been defined that prevents progression to the more aggressive forms of ATLL. Although chronic or smoldering ATLL may respond to zidovudine and interferon, randomized studies have not been conducted. (3) Lymphoma-type ATLL is characterized by ≤1% abnormal circulating T lymphocytes and features of non-Hodgkin lymphoma. (4) Acute-type ATLL includes the remaining patients. Even with optimal therapy, the median survival of lymphoma and acute-type ATLL is <1 year. Lymphoma and acute types of ATLL are the most common presenting subtypes. Other major prognostic factors include performance status, age, the presence of more than three involved lesions, and hypercalcemia.



**Figure 7.3** Clinical manifestation of adult T-cell leukemia/lymphoma. **A,B:** Infiltration of malignant T lymphocytes into the skin. **C:** Lytic bone lesions seen on lateral skull x-ray. **D:**

“Flower cells” in the blood.

Combination chemotherapy for lymphoma or acute-type ATLL with the infusional etoposide, prednisone, vincristine, and doxorubicin (EPOCH) regimen or the LSG-15 regimen results in complete remission rates of 15% to 40%. However, responses are short lived, with <10% of patients free of disease at 4 years. The addition of anti-CCR4 antibody, mogamulizumab, improves response rates. The combination of interferon and zidovudine with or without arsenic trioxide may result in the remission of acute but not lymphoma subtypes. Lenalidomide has activity in relapsed or recurrent ATLL. Allogenic hematopoietic stem cell transplantation may result in long-term, disease-free survival for patients with complete or near complete remission of disease, although infectious complications have been notable in these studies.

# HEPATITIS VIRUSES

The earliest record of an epidemic caused by a hepatitis virus was in 1885, occurring in individuals vaccinated for smallpox with lymph from other people. The cause of the epidemic, HBV, was not identified until 1966, when Blumberg discovered the *Australian antigen* now known to be the hepatitis B surface antigen (HBsAg). This was followed by the discovery of the virus particle by Dane in 1970. In the early 1980s, the HBV genome was sequenced and the first vaccines were tested. In the mid-1970s, Alter described cases of hepatitis not due to hepatitis A or B viruses, and the suspected agent was designated non-A, non-B hepatitis virus, now known as HCV. In 1987, Houghton used molecular cloning to identify the HCV genome and develop a diagnostic test, which was licensed in 1990. It is now appreciated that, in addition to causing hepatitis and cirrhosis, HBV and HCV cause hepatocellular carcinoma (HCC), which constitutes approximately 90% of all primary liver cancer cases.

According to the World Health Organization, roughly 257 million people are currently living with HBV infection and 71 million people have chronic HCV infection. It is estimated that 880,000 deaths per year are attributable to HBV-induced liver disease or HCC and an additional 350,000 to 500,000 people die of HCV-related liver disease or HCC. HBV and HCV are the leading cause of liver cancer in the world, accounting for almost 80% of cases. In the United States, Europe, Egypt, and Japan, more than 60% of HCC cases are associated with HCV, and 20% are related to HBV and chronic alcoholism. In Africa and Asia, 60% of HCC is associated with HBV, 20% is related to HCV, with the remainder related to other risk factors, such as alcoholism and dietary exposure to fungal aflatoxin. Liver cancer is the sixth most common cancer worldwide and is the second most common cause of cancer death in men.

In Asia and Africa, up to 70% of individuals have serologic evidence of current or prior HBV infection, and 8% to 15% of these subjects have a chronic active infection. Rates of HCV infection of >3.5% occur in Central and East Asia, North Africa, and the Middle East. In the United States, 0.8 to 1.4 million individuals are infected with HBV, and 3.2 million with HCV. The incidence of HCC in the United States tripled between 1975 and 2005, particularly in African American and Hispanic males.

HBV is transmitted primarily through exposure to infected blood, semen, and other body fluids, whereas HCV is transmitted primarily by blood or sexual contact. Acute HCV infection causes mild and vague symptoms in about 15% of individuals and resolves spontaneously in 10% to 50% of cases. Liver enzymes are normal in 5% to 50% of individuals with chronic HCV infection. After 20 years of chronic HCV infection, the likelihood of cirrhosis is 10% to 15% for men and 1.5% for women. Cofactors that increase the likelihood of cirrhosis are coinfection with both hepatitis viruses, persistently high levels of HBV or HCV viremia, HBsAg, certain viral genotypes, schistosomiasis, HIV, alcoholism, male gender, advanced age at the time of infection, diabetes, and obesity.

## Hepatitis B Virus

HBV is an enveloped DNA virus that is a member of the viral family *Hepadnaviridae*. HBV has a strong preference for infecting hepatocytes. Although HBV is not associated with extrahepatic disease, small amounts of viral DNA can be found in kidney, pancreas, and mononuclear cells. The viral genome is a relaxed circular partially double-stranded DNA of 3.2 kb. The genome exists as an episomal covalently closed circular double- stranded DNA molecule in the nucleus of infected cells, although chromosomal integration of viral genomic sequences can occur during cycles of hepatocyte regeneration and proliferation. In addition to 40 to 42 nm virions,

HBV-infected cells also produce noninfectious 20-nm spherical and filamentous subviral particles. The viral genome encodes four open reading frames. The presurface–surface (preS-S) region encodes three proteins from different translational initiation sites; these include the S (HBsAg), M (or pre-S2), and L (or pre-S1) proteins. The L protein is responsible for receptor binding and virion assembly. The precore–core (preC-C) region encodes the HBcAg and HBeAg. The P region encodes the viral polymerase, and the X (HBx) protein modulates host-cell signal transduction.

After infection, the viral genome is transcribed by host RNA polymerase II, and viral proteins are translated. Nucleocapsids assemble in the cytosol, incorporating a molecule of pregenomic RNA into the viral core, where reverse transcription occurs to produce the double-stranded DNA viral genome. Viral cores are enveloped with intracellular membranes and viral L, M, and S surface antigens, which are exported from the cells.

HBV replication is not cytotoxic. Instead, liver injury is due to the host immune response, primarily T-cell and proinflammatory cytokine responses. Chronic HBV carriers exhibit an attenuated virus-specific T-cell response, although a vigorous humoral response is typically evident. About 5% of infections in adults and up to 90% of infections in neonates result in a persistent infection, which may or may not be associated with symptoms and elevated serum aminotransferase levels. Immunosuppressed individuals have a higher likelihood of a persistent infection. About 20% of persistently infected individuals develop cirrhosis.

With acute infection, viral titers of 109 to 1010 virions per milliliter are present, whereas persistently infected individuals have somewhat lower levels, ranging from 107 to 109 virions per milliliter. The resolution of infection, which is associated with declining viral DNA titers, is observed at a rate of 5% to 10% per year in persistently infected individuals. However, even subjects who have resolved the infection continue to have very low levels of viral DNA (103 to 105 copies per milliliter) for most of their lives.

Chronic HBV infection can be managed with alpha interferon but the treatment cures only 3% to 15% of infected individuals and has severe side effects. Nucleoside/nucleotide analogs, such as lamivudine, telbivudine, entecavir, adefovir, and tenofovir, inhibit HBV reverse transcriptase and limit HBV replication in a majority of patients. Entecavir and tenofovir are both effective at inducing viral suppression and may be used in combination in patients with high HBV DNA load or multidrug resistance. Because these agents are all associated with some toxicity, current guidelines recommend therapy only when liver disease is clinically apparent, with continued treatment for 6 to 12 months after clearance of HBeAg or HBsAg. Although these drugs effectively control HBV, they typically fail to cure the infection due to the long-term persistence of the covalently closed circular double-stranded DNA form of the viral genome. Other nucleoside/nucleotide analogs are currently in clinical trials, as well as a novel form of interferon (IFN)-λ and an inhibitor of virus release. Current results suggest that long-term anti-HBV therapy can reduce the risk of HCC by about 50%.

Hepatitis D virus (HDV) occurs only in individuals coinfected with HBV. HDV is composed a single-stranded circular viral RNA genome of 1,679 nucleotides, a central core of HDAg, and an outer coat with all three HBV envelope proteins. HDV infection results in more severe complications than infection with HBV alone, with a higher likelihood and more rapid progression to cirrhosis and HCC.

## Hepatitis C Virus

HCV is a positive-sense, enveloped single-stranded RNA virus of the *Flaviviridae* family. There are seven genotypes of HCV; in the United States, about 70% of infections are caused by genotype 1. HCV replicates in the cytoplasm and does not integrate into the host cell genome. The viral RNA is 9.6 kb and encodes a single polyprotein of 3,010 amino acids that is proteolytically processed into structural and nonstructural proteins. In addition to the structural roles of the core (C) protein, it has also been reported to affect various host cell functions. The envelope glycoproteins E1 and E2 mediate infectious entry through tetraspanin CD81 and other receptors on hepatocytes and B lymphocytes.

HCV nonstructural proteins NS2, NS3, NS4A, NS4B, NS5A, NS5B, and p7 are required for virus replication and assembly. NS2 is a membrane-associated cysteine protease. NS3 is a helicase and NTPase that unwinds RNA and DNA substrates. The complex of NS3 with NS4A forms a serine protease. NS4B induces the formation of a membranous web associated with the viral RNA replicase. NS5A is an RNA-binding phosphoprotein, whereas NS5B is the RNA-dependent RNA polymerase. The p7 protein forms a cation channel in infected cells that has a role in particle maturation and release.

Older treatments for HCV infection utilized 24 to 48 weeks of pegylated IFN-α and ribavirin. Treatment with IFN and ribavirin alone produces sustained virologic response (SVR) in 70% to 80% of subjects with genotype 2 or 3 infections. Although patients with SVR show reduced risk of HCC, a 10-year follow-up found a

2.5% risk of HCC in such patients. Patients with cirrhosis have been estimated to have a 1% annual risk of HCC after successful SVR. The results illustrate the utility of detecting HCV infection prior to the development of cirrhosis and show the importance of ongoing monitoring of patients, even after SVR.

Treatment options for HCV infection have changed dramatically with the advent of the NS5B polymerase inhibitor sofosbuvir and newer direct-acting antiviral therapies. The new treatment regimens show SVR rates

>90% against all HCV genotypes after 8 to 12 weeks and have markedly fewer side effects than IFN-based treatments. The current cost of a 12-week course of sofosbuvir treatment is $84,000, and in 2015, it was the number two best-selling pharmaceutical in the U.S. market. The high cost of HCV treatment has limited its uptake, particularly in underresourced countries.

## Hepatitis Virus Pathogenesis

HBV and HCV depress innate immune responses by inhibiting Toll-like receptor signaling through effects of HBx and NS3-4A. In addition, HCV C inhibits the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signaling, and NS5A and E2 inhibit IFN signaling. Through an undefined mechanism, HBV can inhibit JAK-STAT signaling as well.

HBV and HCV induce HCC by direct and indirect mechanisms. Both HBV and HCV encode proteins that have pro- and antiapoptotic properties. High levels of HBx block activation of the NF-κB pathway, whereas HCV C and NS5A block apoptosis by the activation of AKT and NF-κB, respectively. The C and NS5A proteins may also induce epithelial–mesenchymal transition, which is important for liver fibrosis, through effects on transforming growth factor β and Src signaling. Mice transgenic for NS5A develop steatosis and HCC.

HBx and HCV C are associated with mitochondria, where they trigger oxidative stress that induces apoptosis. In addition, HBs and HBx and NS3-4A alter calcium signaling and increase reactive oxygen species, which trigger endoplasmic reticulum (ER) stress, an unfolded protein response, and the production of proinflammatory cytokines that induce collagen synthesis and fibrosis. Autophagy is triggered by both viruses to restore ER integrity, which promotes cell survival and viral persistence.

HBV and HCV also disrupt tumor suppressor proteins. HCV NS5B recruits a ubiquitin ligase protein to modify pRB and induce its degradation, whereas HBx and HCV C proteins both inhibit p16INK4a and p21 cell cycle inhibitors, which leads to inactivating phosphorylation of pRB. The HBx and HCV C, NS3, and NS5A proteins deregulate p53 tumor suppressor activity by compromising p53-mediated DNA repair. HBV and HCV also induce alterations in micro-RNAs that are partially responsible for cell cycle effects.

Although not part of the normal virus replication cycle, the tendency of HBV DNA to integrate within the host cell chromosomes also contributes to the pathogenesis of HBV-associated HCC. In most HCC tumor cells, HBV replication is extinguished, and integration at certain sites provides a growth or survival advantage, leading to tumors that are clonal with respect to viral integration. Whole-genome sequencing studies have identified a number of cellular loci, including *TERT* and *MLL*, where HBV integration is associated with HCC.

Both HBV and HCV promote characteristics of cancer stem cells. HBx promotes the expression of Nanog, Kruppel-like factor 4, octamer-binding transcription factor 4, and Myc. These markers are also induced by HBV- and HCV-induced hypoxia and hypoxia-induced factors.

## Clinical Characteristics and Treatment of Hepatitis Virus–Associated Malignancies

HBV and HCV infections are diagnosed by serologic assays and/or antigen assays in the case of HBV. Quantitative polymerase chain reactions are utilized to measure virus load. No vaccine has been identified that protects against HCV because infections consist of a genetically heterogeneous “swarm” of virus sequences, some of which escape neutralization.

Recombivax HB, which utilizes a recombinant HBsAg produced in yeast cells, has been available for HBV prevention for more than 30 years. The vaccine is more than 90% effective for individuals younger than 40 years of age, and protection lasts at least three decades. The vaccine is recommended for infants, adolescents aged 11 to 15 years, and adults with potential risk of HBV exposure. A newer combination vaccine, Twinrix, offers protection against both hepatitis A virus and HBV. Factors associated with HBV vaccination failure in adults include increased age, obesity, smoking, diabetes, end-stage renal disease, HIV infection, alcoholism, or recipients of liver or kidney transplantation. In these cases, use of a higher dose of the vaccine can improve responses. Recent clinical trials have suggested that improved adjuvants could allow fewer doses and improved

seroprotection rates.

Because an early diagnosis of HCC is a key to successful treatment, there has been extensive research on surveillance techniques in HBV- and HCV-infected individuals. The Centers for Disease Control and Prevention recommends that all individuals born between 1945 and 1965 be tested for HCV infection. The American Association for the Study of Liver Diseases, as well as the European and Asian Pacific Associations for the Study of the Liver, endorse surveillance in HCV-infected individuals with cirrhosis using ultrasound every 6 months. Viral eradication does not fully eliminate the risk of HCC and continued surveillance is still recommended in cirrhotic patients.

Therapeutic options for HCC are determined not only by the number and size of HCC nodules, as well as the presence or absence of vascular invasion and metastases, but also by liver function and the presence or absence of portal hypertension. HCC amenable to liver transplantation is usually defined as either one tumor measuring

≤50 mm in diameter or two to three tumors measuring ≤30 mm in diameter without vascular extension or metastasis (Milan criteria). Up to 30% of all cases of HCC present with multiple nodules of HCC, suggesting a field carcinogenesis effect of HBV and HCV. HBV- and HCV-infected patients may have a lower survival than noninfected patients after liver transplantation. Hepatitis B immune globulin and NAs are recommended for reinfection prophylaxis in the posttransplant period for HBV-infected individuals. Antiviral therapy is recommended for HCV-infected patients undergoing liver transplantation.

Reactivation of HCV can occur with chemotherapy or monoclonal antibody–based immunosuppressive therapies but is less frequent than HBV reactivation. Individuals who appear to have cleared an HBV infection and who have an undetectable viral load can experience HBV reactivation on rituximab therapy. Monitoring hepatic function and virus load is indicated during chemoimmunotherapy of HBV- or HCV-positive patients. Although there is controversy regarding the role of virus screening for patients undergoing chemotherapy, antiviral therapy is recommended for high-risk HBV-infected patients undergoing chemoimmunotherapy, such as rituximab-based chemotherapy regimens.

An association between HCV and B-cell non-Hodgkin lymphoma has also been demonstrated in highly endemic geographic areas. Diffuse large B-cell lymphoma, marginal zone lymphomas, mixed cryoglobulinemia, and lymphoplasmacytic lymphomas are the histologic subtypes most frequently associated with HCV infection. Antiviral treatment with IFN-α with or without ribavirin has been effective in the treatment of HCV-infected patients with indolent lymphoma, but rarely in individuals with aggressive lymphomas.