The History of Tumor Virology

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Abstract

In the century since its inception, the field of tumor virology has provided groundbreaking insights into the causes of human cancer. Peyton Rous founded this scientific field in 1911 by discovering an avian virus that induced tumors in chickens; however, it took 40 years for the scientific community to comprehend the effect of this seminal finding. Later identification of mammalian tumor viruses in the 1930s by Richard Shope and John Bittner, and in the 1950s by Ludwik Gross, sparked the first intense interest in tumor virology by suggesting the possibility of a similar causal role for viruses in human cancers. This change in attitude opened the door in the 1960s and 1970s for the discovery of the first human tumor viruses—EBV, hepatitis B virus, and the papillomaviruses. Such knowledge proved instrumental to the development of the first cancer vaccines against cancers having an infectious etiology. Tumor virologists additionally recognized that viruses could serve as powerful discovery tools, leading to revolutionary breakthroughs in the 1970s and 1980s that included the concept of the oncogene, the identification of the p53 tumor suppressor, and the function of the retinoblastoma tumor suppressor. The subsequent availability of more advanced molecular technologies paved the way in the 1980s and 1990s for the identification of additional human tumor viruses—human T-cell leukemia virus type 1, hepatitis C virus, and Kaposi's sarcoma virus. In fact, current estimates suggest that viruses are involved in 15% to 20% of human cancers worldwide. Thus, viruses not only have been shown to represent etiologic agents for many human cancers but have also served as tools to reveal mechanisms that are involved in all human malignancies. This rich history promises that tumor virology will continue to contribute to our understanding of cancer and to the development of new therapeutic and preventive measures for this disease in the 21st century. [Cancer Res 2008;68(19):7693–706]

Early Theories on Cancer Origins

Cancer as a disease was recognized several thousand years ago. Different theories on the cause of cancer found favor over time, but none considered an infectious etiology until the 20th century.

The earliest evidence for human cancer comes from bone tumors found in 4 million–year-old fossilized hominid remains and from nasopharyngeal carcinomas and osteogenic sarcomas seen in ancient Egyptian mummies from 3000 BCE (1). Some of the first written accounts of human cancer were recorded in the Babylonian *Code of Hammurabi* (1750 BCE), ancient Egyptian papyri (1600 BCE), the Chinese *Rites of the Zhou Dynasty* (1100–400 BCE), and the ancient Indian *Ramayana* manuscript (500 BCE). In ancient Egypt, intellectual power was primarily restricted to priests who claimed to be direct recipients of divine knowledge, so it is not surprising that writings of the time attributed the etiology of diseases such as cancer to the "will of Gods" (1, 2).

The ancient Greek civilization, on the other hand, is credited with freeing medicine from the bonds of religion (2–4). Rather than accepting religious dogma, Hippocrates (460–370 BCE) used systematic observation and logical thinking to propose the humoral theory of cancer. Based on teachings by the Greek philosopher Empedocles who believed that air, water, earth, and fire were the four cardinal elements of the universe, Hippocrates theorized that the human body contains a mixture of the four biological counterparts—blood, phlegm, yellow bile, and black bile. He proposed that a proper balance of these four fluids results in a state of health whereas an imbalance produces disease, with cancer specifically stemming from an accumulation of excess black bile at the afflicted body site. With the decline and fall of ancient Greece, the humoral theory of cancer passed on to the Romans and was accepted by the influential Roman physician Galen. This theory remained unchallenged for over 1,300 years. Knowledge stagnated during this extended period of time because religious beliefs and convictions prohibited the study of the body, including carrying out autopsies.

In 1540, the failure of Andreas Vesalius to confirm the existence of black bile led to the demise of the humoral theory and the eventual emergence of the related lymph theory of cancer (2, 4). With the discovery of lymph by Gasparo Aselli in 1622 and the demonstration of blood circulation by William Harvey in 1626, lymph replaced black bile as one of the cardinal biological liquids. Based on this new information, Frederick Hoffman and George Stahl proposed in 1695 that life consists of continuous and appropriate movement of body fluids, such as blood and lymph, through solid parts. The postulated source of this fluid movement was God acting through a mystical force called *anima*. The lymph theory further contended that benign tumors were caused by local coagulation of lymph leaked from lymphatic vessels, whereas malignant cancers instead arose from the fermentation and degeneration of lymph. This theory dominated medical thinking for nearly 150 years but was eventually abandoned due to a lack of confirmatory evidence.

The 19th century saw the birth of scientific oncology (2, 4). Due to the invention of the compound microscope in 1590 and achromatic lenses in the 1830s, the study of cancer could be conducted at the microscopic level rather than the gross level as was done in earlier times. With these important technical advancements, Johannes Müller, Rudolf Virchow, and Karl von Rokitansky established the revolutionary view that cancer is a disease of cells and not lymph. Nonetheless, Müller theorized that tumor cells arise from budding elements, called blastema, scattered between normal tissue components. It was Müller's student, Rudolph Virchow, who disproved the blastema theory by demonstrating that cancer cells

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doi:10.1158/0008-5472.CAN-08-3301

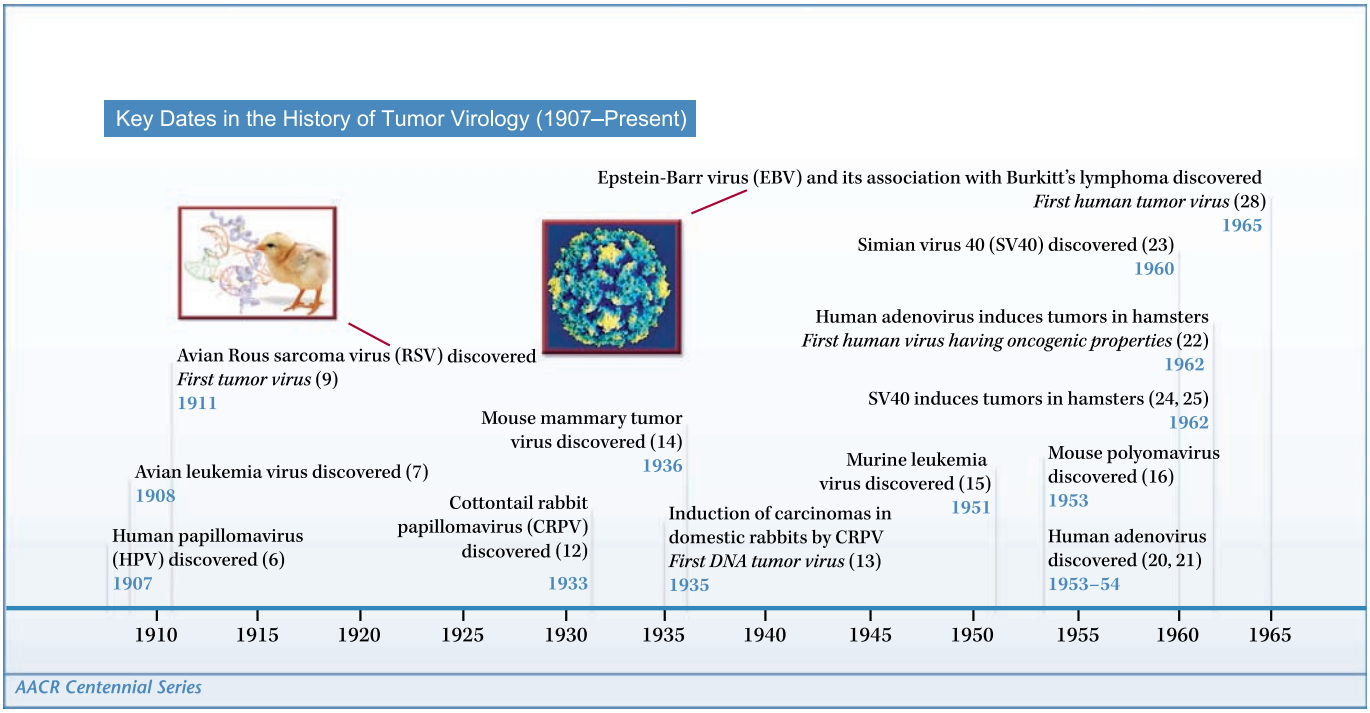

Figure 1. Timeline of advances in tumor virology.

are derived from other cells, although he falsely held the view that metastatic cancers are spread by a liquid. Karl Thiersch went on to show that metastatic cancers arise not from a liquid but from the spread of malignant cells. Consequently, in the 19th century, the lymph and blastema theories were disproved and replaced by the modern cellular theory of cancer.

During the 19th century, several hypotheses were put forth to explain the etiology of cancer cells (2, 4). Virchow postulated that chronic irritation promoted cancer, whereas Hugo Ribbert thought that trauma was the cause of cancer, despite a failure to induce tumors in experimental animals by injury. With the development of microbiology in the same century, accumulating evidence began to implicate infections with various bacteria, yeasts, fungi, and protozoa in the development of cancer. In fact, a Nobel Prize was awarded in 1926 for scientific research documenting a nematode worm that provoked stomach cancer in rats. The failure of rigorous experimentation to verify this and other work implicating microbes in cancer, however, eventually led to an unfortunate dogmatic prejudice that this disease does not have an infectious origin. This biased viewpoint persisted for a half century until studies with a newly discovered class of infectious agent—the virus—would yield the first key clues about molecular mechanisms that trigger the development of human cancer.

The Early 20th Century: Avian Tumor Viruses

Near the close of the 19th century, Dimitrii Ivanofsky and Martinus Beijerinck became the fathers of the new field of virology by showing that an infectious pathogen of tobacco plants not only retained infectivity after passage through a filter capable of removing bacteria but also failed to replicate in cell-free culture (5). These novel observations led to the discovery of a new class of "filterable" infectious agents called viruses, which are substantially smaller than any bacteria and are able to replicate only in living tissues. This landmark discovery was rapidly followed by the identification of the first animal virus (foot-and-mouth disease virus) and the first human virus (yellow fever virus).

Shortly thereafter in 1907, the Italian physician Giuseppe Ciuffo showed a viral etiology for human warts when cell-free filtrates from such lesions were shown to transmit the disease (ref. 6; Fig. 1). The relevance of this finding to the field of tumor virology, however, would not be appreciated for another 70 years when wart viruses, now known as papillomaviruses, were linked to human cancer. In 1908, two Danish scientists, Vilhelm Ellermann and Olaf Bang, reported that upon inoculation into healthy chickens, a cell-free filtrate of chicken leukemia cells passed on the disease (7). Unfortunately, because current knowledge was insufficiently advanced to recognize the cancerous nature of leukemia, the filterable agent (avian leukemia virus) present in the leukemia cell extracts was not designated as a tumor virus (8). In fact, another 40 years would pass before leukemia was generally accepted as a cancer of bone marrow–derived cells. Thus, the important discovery made by Ellermann and Bang went largely unnoticed for a major part of the 20th century.

In 1911, Peyton Rous at the Rockefeller Institute showed that a transplantable, spontaneous spindle cell sarcoma derived from a Plymouth Rock chicken could be transmitted to healthy chickens using filtered cell-free tumor extracts (ref. 9; Fig. 2). Contrary to the leukemia studied by Ellerman and Bang, the avian sarcoma induced by Rous sarcoma virus (RSV), as it became called, was shown a year earlier by Rous to represent a genuine cancer, similar to malignant solid tumors seen in mammals (10). This led to the designation of RSV as the first known tumor virus and to the recognition of a new paradigm in cancer research—the virus-induced cancer. As RSV has an RNA genome packaged into virus particles, it was also the first known RNA tumor virus.

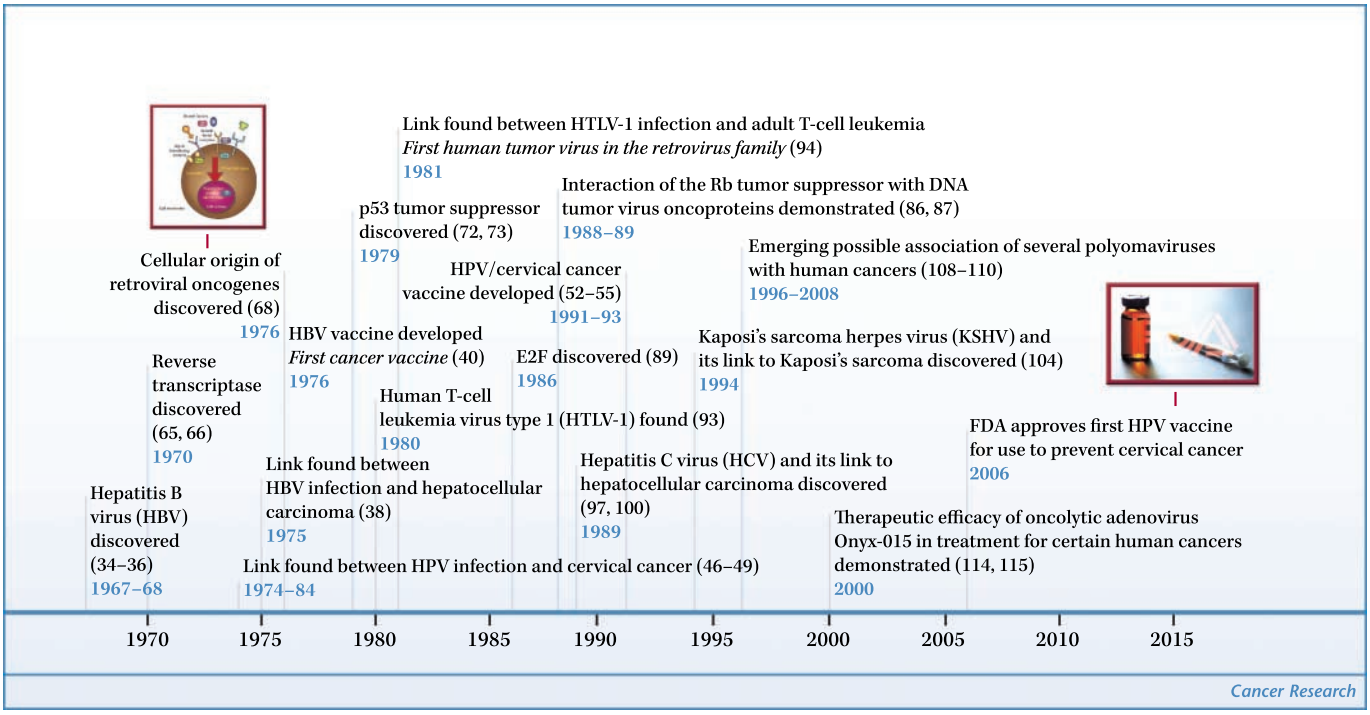

Figure 1 *Continued.*

Unfortunately, this seminal discovery by Rous was not embraced by most contemporary scientists who sought to dismiss the findings by arguing that RSV-induced sarcomas represented a form of infectious granuloma rather than a cancer, that the tumor cell filtrates were not cell-free, that cell fragments or submicroscopic cells passed through the filters and formed daughter cells capable of generating new tumors, and that chickens were too distantly related to humans to provide a useful model for human disease (8, 11). Due to this rejection, Rous terminated research on RSV only a few years after publishing his report. In fact, not until 1966, 55 years after publication of the first RSV article, was Rous finally awarded the Nobel Prize for his groundbreaking discovery. The magnitude of this achievement is underscored by the fact that, within 10 years of Rous receiving the Nobel Prize, studies of RSV would lead to additional landmark discoveries in the cancer field—reverse transcription and the cellular origin of viral oncogenes.

The 1930s to 1960s: Mammalian Tumor Viruses

Despite persistent dogma dismissing a possible role for viruses in cancer, researchers continued to identify new tumor viruses over the next four decades after the discovery of RSV. In 1933, Richard Shope and E. Weston Hurst showed that soluble extracts of warts from wild cottontail rabbits contained a filterable agent, the Shope papillomavirus, capable of transmitting the disease to wild cottontail rabbits (12). In 1935, Peyton Rous and Joseph Beard revealed the tumorigenic potential of this wart virus, now called cottontail rabbit papillomavirus (CRPV), by showing that it failed to replicate and produce warts in the domestic rabbit, a different species than the cottontail rabbit, and instead induced the formation of skin carcinomas after an extended time period (13). Unlike the RNA tumor viruses, CRPV has a DNA genome packaged into virus particles, so it represented the first known DNA tumor virus. During the next two decades, CRPV infection of rabbits was an important model for the study of viral tumorigenesis, but the most important ramification of the work would only be realized some 30 years later when human papillomavirus (HPV) infection was linked to human cancer.

In 1936, John Bittner reported that certain mouse strains were highly prone to develop mammary tumors whereas other strains were resistant (14). Moreover, if cancer-resistant newborn mice suckled from a cancer-prone mother, they would show a high incidence of mammary tumors. Bittner showed that an infectious filterable agent, now called mouse mammary tumor virus, was present in the milk of cancer-prone mice and transmitted the disease. These findings prompted numerous attempts over the next decade to show a virus etiology for other cancers of mice. Although these efforts initially proved unsuccessful, studies by Ludwik Gross (Fig. 2) eventually led to the identification of the first mouse leukemia virus (murine leukemia virus) in 1951 and a mouse virus that induced a variety of solid tumors (mouse polyomavirus) in 1953 (15, 16). Nevertheless, for several years, critics of tumor virology remained highly skeptical about the overall significance of this work until other researchers, such as Arnold Graffi, Charlotte Friend, and John Moloney, similarly reported the identification of mouse leukemia viruses (17–19). All of these mouse viruses were RNA tumor viruses, except for mouse polyomavirus, which was a DNA tumor virus.

During the same time period, DNA tumor viruses of human and simian origin were also discovered. In 1953 and 1954, Wallace Rowe, Maurice Hilleman (Fig. 2), and colleagues discovered human adenovirus in explants of adenoid and tonsil tissue and showed that this virus caused acute respiratory illness in people (20, 21). In 1962, John Trentin and colleagues reported that certain human adenoviruses are tumorigenic in experimentally infected animals (22). Human adenovirus importantly represented the first known

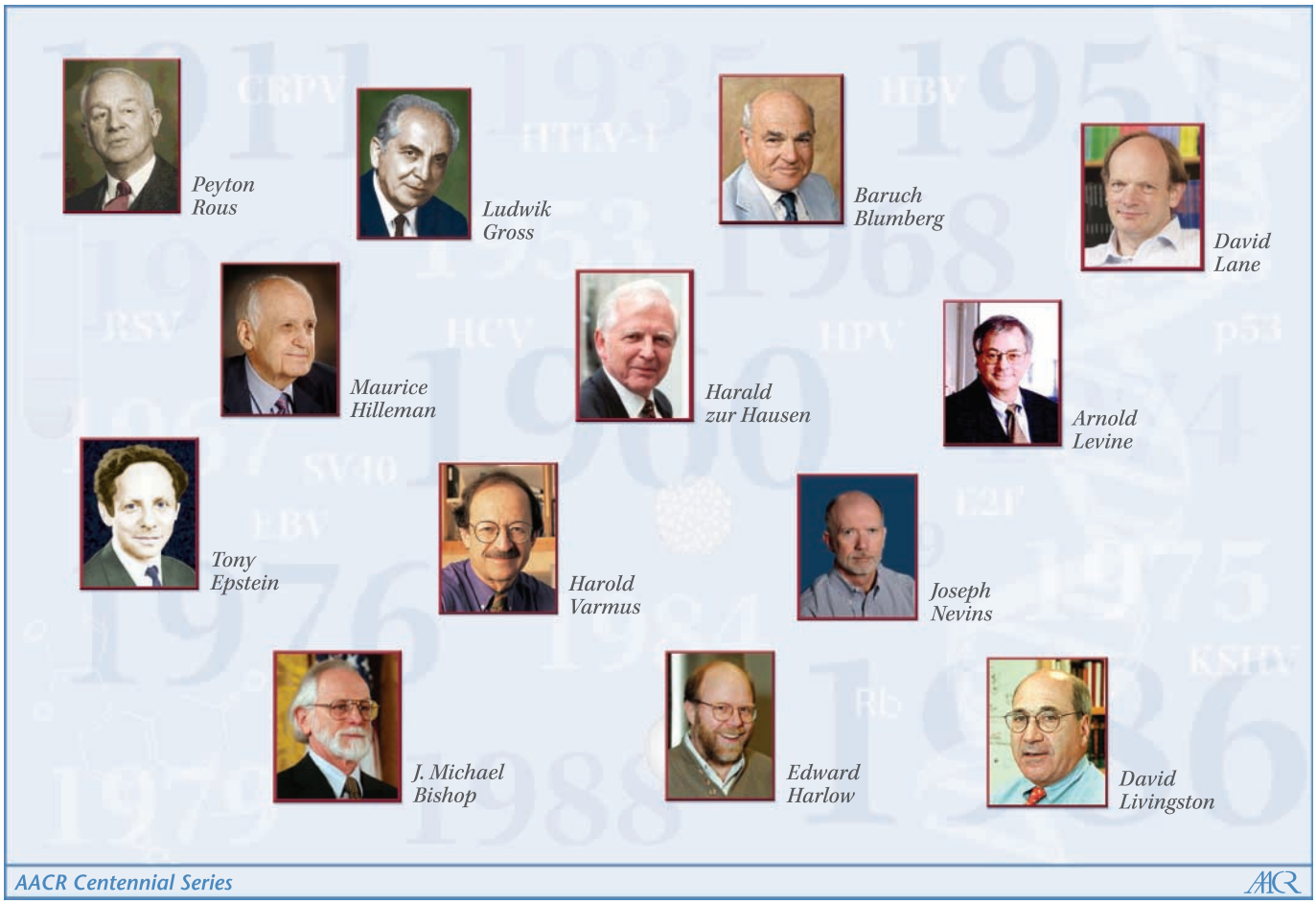

Figure 2. Photographs of Peyton Rous, Ludwik Gross, M. Anthony Epstein, Baruch Blumberg, Harald zur Hausen, Maurice Hilleman, J. Michael Bishop, Harold Varmus, David Lane, Arnold Levine, Ed Harlow, David Livingston, and Joseph Nevins. These experts represent some of the major investigators in the field of tumor virology.

human virus capable of inducing cancer, albeit under experimental conditions. In 1960, Ben Sweet and Maurice Hilleman identified SV40 in rhesus monkey kidney cell cultures used for the production of the Salk poliovirus vaccines (23). Two years later, Bernice Eddy, Maurice Hilleman, and colleagues also showed tumorigenic potential for SV40 (24, 25).

With this accumulating body of evidence suggesting a potential role for viruses in cancer, the scientific community finally began to understand and appreciate the significance of research on tumor viruses, including the classic avian tumor viruses studied by Ellerman, Bang, and Rous. In fact, this new attitude together with the identification of many new tumor viruses strengthened the idea that human tumors might be caused by viruses, leading to the creation of the U.S. Special Virus Cancer Program in 1964, which for the next 13 years invested major public resources in a search for human cancer viruses.

The 1960s to 1970s: Human Tumor Viruses

EBV: the first human tumor virus. During the 1960s, the general acceptance of the concept that viruses could cause cancer in animals spawned an intense interest to identify viruses similarly associated with human malignancies. Numerous attempts to isolate human tumor viruses, however, were disappointingly negative, which raised serious doubts about the effort. Nonetheless, the quest would soon be fulfilled.

In the 1950s, Denis Burkitt, a British surgeon working in East Africa, was the first to describe a novel childhood tumor, now known as Burkitt's lymphoma (26, 27). Interestingly, cases of this disease, which is a cancer of B cells that normally produce antibodies, were found to closely follow the African malarial belt. Based on the expectation that viruses would be found to promote some human cancers, coupled with the known transmission by mosquitoes of not only the malaria parasite but also a large group of viruses (i.e., arboviruses), Burkitt suspected that a virus, perhaps transmitted by an arthropod vector, might be the etiologic agent for the lymphoma. This novel idea captured the attention of virologists and, in 1965, Tony Epstein (Fig. 2), Yvonne Barr, and colleagues succeeded in establishing cell lines derived from Burkitt's lymphomas and in visualizing herpesvirus-like particles in a small percentage of the cells by using electron microscopy (28). Extending this landmark discovery, Werner and Gertrude Henle proved that this virus was biologically and antigenically distinct from other known human herpesviruses (29). This new virus was named EBV.

The proposed role of EBV in Burkitt's lymphoma, however, was again met with great skepticism due to various technical and theoretical arguments (8). In addition, given the specific link of

EBV to an African tumor, it was quite unexpected when subsequent seroepidemiologic studies showed a worldwide distribution for this virus in human populations, with >90% of adults scoring positive for serum antibodies (30). Nonetheless, overwhelming evidence now supports the contention that EBV plays a central role in African Burkitt's lymphoma (27, 31). First, EBV is detected in the vast majority of African Burkitt's lymphomas. Second, EBV is the etiologic agent for infectious mononucleosis, an acute infection of B cells that can result in B-cell lymphomas in immunosuppressed individuals. Third, experimental EBV infection of the marmoset, a small primate, similarly induces B-cell lymphomas. Fourth, in addition to a causal role in Burkitt's lymphoma, EBV infection is strongly associated with nasopharyngeal carcinoma, posttransplant lymphomas, and some Hodgkin's lymphomas, and has been tentatively linked to several other types of human malignancies. Consequently, EBV represented the first known human tumor virus (Table 1).

The fact that an arthropod vector does not transmit EBV infections to people and that the majority of individuals infected with EBV do not develop EBV-associated cancers led to the proposal that genetic or environmental factors (e.g., insect-borne parasitic infections like malaria, young age, status of immune system, and nutrition) also play a crucial role in the development of Burkitt's lymphoma (27, 31). Also notable is that nearly all Burkitt's lymphoma cells not only contain the EBV genome but also a chromosomal translocation that results in abnormally high expression of the cellular *myc* oncogene. These collective observations indicate that EBV is necessary but not sufficient to induce lymphoma and that additional cofactors, such as the *myc* translocation and an unknown environmental factor possibly transmitted by insects, are also required. Clearly, our understanding of Burkitt's lymphoma remains incomplete. Nonetheless, the classic story of this disease illustrates the fundamental concept of tumor virology that, as opposed to representing complete carcinogens, viruses generally act as initiating or promoting factors of the carcinogenic process, consistent with the principle that cancer development occurs not by a single event but rather by the accumulation of cooperating events.

Hepatitis B virus: link to human hepatocellular carcinoma and development of the first human cancer vaccine. Serum hepatitis was a recognized disease as early as 1895 when jaundice and hepatitis-like symptoms were reported in German shipyard workers who had received a contaminated smallpox vaccine. Throughout the 20th century, episodic cases of hepatitis were associated with improperly sterilized syringes and needles and, by 1937, it was clear that a virus present in the blood or blood products caused this disease (32). In 1965, Baruch Blumberg (Fig. 2) reported attempts to correlate inherited polymorphic traits in different geographic areas of the world with particular disease patterns (33). His approach was to screen for polymorphisms in thousands of blood samples collected from diverse populations using sera from multiply-transfused hemophilia patients, who were postulated to have generated antibodies reactive to many unique protein polymorphisms. During this study, Blumberg found that one blood sample from an Australian aborigine contained an antigen that reacted specifically with an antibody in serum from an American hemophilia patient. The first clue about the nature of the Australia (Au) antigen, as it was called, came from an incident in which a technician working with human sera contracted hepatitis (27). After but not prior to the illness, her serum was Au antigen–positive.

In 1967 and 1968, Baruch Blumberg, Alfred Prince, Kazuo Okochi, and Seishi Murakami published seminal studies showing that blood from patients with serum hepatitis specifically contained the Au antigen (34–36), which would eventually prove to be the surface antigen of a hepadnavirus called hepatitis B virus (HBV), the etiologic agent of serum hepatitis. This major breakthrough paved the way for intensive study of the disease (37). In adults, the primary HBV infection either is asymptomatic or causes acute hepatitis, which generally resolves by immune-mediated clearance of the virus from the liver and blood. In ~5% of infected adults, however, the primary infection fails to resolve,

Table 1. Accepted and candidate human tumor viruses and selected animal model tumor viruses

Virus family	Causal role in human cancer		Animal models
	Accepted	Potential	
Adenoviridae		Subgroups B, C, D	Multiple serotypes
Flaviviridae	HCV		
Hepadnaviridae	HBV		WHV, GSHV
Herpesviridae			
γ-Herpesviruses	EBV, KSHV/HHV-8		HVS, MuγHV
Papillomaviridae	HPV (high-risk types)	HPV (other types)	CRPV, BPV
Polyomaviridae		SV40, BKV, JCV, MCPyV	SV40, MuPyV
Retroviridae			
Simple		XMRV, HERVs, HMTV	ALV/ASV, MLV/MSV, FeLV/FeSV, MMTV
Complex	HTLV-1		BLV

NOTE: Adapted from J. Butel (57).

Abbreviations: ALV/ASV, avian leukosis-sarcoma virus group; BLV, bovine leukemia virus; BKV, BK virus; BPV, bovine papillomavirus; FeLV/FeSV, feline leukemia-sarcoma virus group; GSHV, ground squirrel hepatitis virus; HERVs, human endogenous retroviruses; HMTV, human mammary tumor virus; HVS, herpesvirus saimiri; JCV, JC virus; MCPyV, Merkel cell polyomavirus; MLV/MSV, murine leukemia-sarcoma virus group; MMTV, mouse mammary tumor virus; MuγHV, murine γ-herpesvirus; MuPyV, murine polyoma virus; WHV, woodchuck hepatitis virus; XMRV, xenotropic murine leukemia virus–related virus.

and such individuals develop long-term chronic infections characterized by active viral replication in liver cells and the presence of virus in the blood. In contrast, up to 90% of those infected as neonates or as young children develop chronic infections. It is estimated that there are 350 million chronic carriers of HBV worldwide. Further illustrating the magnitude of this problem, chronic infections account for most of the morbidity and mortality caused by HBV.

In 1975, Blumberg and colleagues discovered a link between chronic HBV infection and hepatocellular carcinoma (HCC; ref. 38), a typically fatal malignancy. Later, a careful prospective study conducted in Taiwan by R. Palmer Beasley and colleagues showed that chronic HBV infection is associated with a 100-fold increased risk for HCC compared with uninfected individuals (39). Thus, like EBV, HBV is also a human tumor virus (Table 1). Underscoring the effect of this discovery, it is now recognized that HBV causes most cases of HCC, which is among the most common cancers in the world, placing HBV in the first rank among known human carcinogens (31). In this regard, estimates indicate that HBV-induced HCC is associated with >300,000 deaths per year worldwide. Reminiscent of EBV-induced lymphomas, the risk of developing HBV-induced liver cancer is substantially increased by certain cofactors, in particular, other hepatotoxins (e.g., aflatoxin from peanuts, as well as hepatitis C virus or parasitic infections). In 1976, Blumberg was awarded the Nobel Prize for his landmark discovery of the Au antigen.

The next major milestone came in 1976 when Maurice Hilleman and colleagues at the Merck pharmaceutical company developed the first effective HBV vaccine by devising a strategy for large-scale purification of HBV surface antigen from the serum of chronic HBV carriers (40). Due to cost considerations and potential safety concerns about a human plasma–derived vaccine, a second-generation recombinant HBV surface antigen subunit vaccine was produced in 1980 and is still in use today. Experience indicates that the HBV vaccine protects not only from acute and chronic hepatitis but also from the development of HCC (41). With respect to the latter point, the Taiwan vaccination program of newborn children against HBV infections, introduced in 1986, not only drastically reduced the percentage of chronically HBV-infected children, but also resulted in a measurable decrease in liver cancer incidence (42). In fact, the HBV vaccine was the first vaccine capable of preventing the development of a specific human cancer, thereby representing a landmark achievement in cancer research (Fig. 1).

HPV: link to cervical cancer and development of an additional human cancer vaccine. The viral etiology of human warts was shown at the turn of the 20th century and the first association of papillomavirus infection with cancer was reported in 1935 when Rous and Beard showed that CRPV caused skin carcinomas in domestic rabbits. The apparent benign nature of human warts together with the lack of a tissue culture system to propagate and study papillomavirus, however, considerably slowed research progress in ensuing years (43). Interest in papillomaviruses was reignited in 1959 when a second papillomavirus (bovine papillomavirus) was found to induce malignant tumors in animals (44), and again in 1972, when cell-free extracts of skin papillomatous plaques from individuals with the rare human hereditary syndrome epidermodysplasia verruciformis were shown to promote wart formation upon inoculation into skin (45). Because papillomatous plaques from patients with epidermodysplasia verruciformis can progress to skin carcinomas, the latter finding hinted at a possible link between HPV infections and human cancer.

Based on these observations and emerging evidence linking HPV infection to genital warts, Harald zur Hausen (Fig. 2) first proposed in 1974 that HPV may represent the etiologic agent for cervical cancer of women (46, 47), despite the general belief at the time that sexually transmitted herpes simplex virus type 2 was the likely cause of this disease. In landmark studies published in 1983 and 1984, zur Hausen substantiated his hypothesis by demonstrating the presence of novel types of HPV DNA in cervical cancers (48, 49). Significantly, these two HPV types, HPV16 and HPV18, are now known to be responsible for ~70% of cervical cancers worldwide (50). Although this discovery spawned a rapid expansion of the HPV field, nearly a decade would pass before the causal role of specific HPV types, termed high-risk HPVs, in cervical cancer and its precursor lesions was generally accepted. Evidence now clearly indicates that HPV is a human tumor virus responsible for causing virtually all cases of cervical cancer in women (ref. 51; Table 1). The effect of this finding is underscored when one considers that cervical cancer is the third leading cause of cancer-related deaths in women worldwide, and is the leading cause in many developing countries in which the majority of cervical cancer cases occur. Furthermore, the same high-risk HPVs are also linked to other anogenital cancers, as well as to a subset of head and neck cancers. In fact, HPVs are associated with more human cancers than any other virus, causing in excess of 500,000 cases of cancer per year worldwide (50). Consequently, HPV has emerged as one of the most important risk factors for human cancer.

The success of the HBV vaccine in decreasing the incidence of liver cancer prompted similar efforts to develop a safe HPV vaccine capable of preventing cervical cancer. From 1991 to 1993, Ian Frazer and others provided the initial approach to accomplish this goal by demonstrating that overexpression of HPV structural proteins in cells results in spontaneous assembly of virus-like particles (VLP; refs. 52–55). VLPs consist of only a single structural viral protein and therefore are safe because they lack the infectious and oncogenic HPV genome. Supporting the idea that such VLPs might be used to develop an effective HPV vaccine, vaccination with VLPs derived from dog, rabbit, or cattle papillomavirus was found to provide protection against primary infection by each virus in the respective host (56). Moreover, evidence indicated that immune responses eliminate the majority of primary HPV infections in people and that spontaneous regression of low-grade and high-grade squamous intraepithelial lesions is invariably accompanied by humoral and cellular immune responses against virus-specific antigens (51). In 2005 and 2006, large-scale clinical trials with commercially developed VLP-based HPV vaccines, which include high-risk HPV16 and HPV18 VLPs, conferred immunized women with type-specific protection against HPV infection, as well as associated cervical, vulvar, and vaginal disease (50). Based on current estimates, these HPV vaccines could prevent more than 300,000 cervical cancer cases per year on a global scale.

The 1970s to 1980s: Tools for Discovery of Mechanisms

The discovery that several human viruses were etiologic agents for human malignancies represented a significant achievement in the cancer field. A second type of major contribution by tumor virology came from studies of RNA and DNA viruses (i.e., RSV, SV40, and human adenovirus) that induced tumors in animals and

transformed cells in culture. Due to their relatively small genome sizes and their utility as model systems, these viruses and their oncogenes served as discovery tools to establish molecular paradigms that extended far beyond virology to form the foundation of contemporary cancer biology (57). In particular, such work played a central role in the development of the revolutionary concept that cancer stems from perturbations in cellular factors that positively or negatively regulate normal cell growth.

RNA tumor viruses: discovery of the oncogene concept. As described above, studies of RSV remained outside the mainstream of cancer research for many decades. Despite this setback, the eventual development of quantitative transformation assays, the pock assay on chorioallantoic membranes of chicken embryos in 1938 (58), and the focus assay on cultured cells in 1958 (59), revolutionized studies of RSV and led to rapid advances in the 1960s. Investigators isolated and characterized many different RSV variants, which soon proved useful in demonstrating that cellular transformation and viral replication by RSV were dissociable properties (60). This observation suggested that RSV encoded a cancer-causing gene dispensable for viral replication, and also set the stage for genetic studies to identify the gene. In 1970, Peter Duesberg and Peter Vogt took the first step in this effort by comparing the RNA genomes of two closely related replication-competent RSV variants, one of which could transform cells and the other which could not (61). The results revealed that the transformation-competent RSV variant exhibited a 20% larger RNA genome than did the transformation-defective RSV variant, with the additional sequences representing a contiguous segment near the 3′-end. This cancer-causing gene was named *src* to denote the type of connective tissue tumor (sarcoma) elicited by RSV in chickens.

Several years earlier, Howard Temin had shown that productive replication of RSV, an RNA tumor virus, unexpectedly depended on both DNA replication and DNA-dependent RNA synthesis (transcription; refs. 62, 63). This information, coupled with the known capacity for stable maintenance of RSV in a nonreplicating state in cells, led Temin to advance the provirus hypothesis wherein the life cycle of an RNA tumor virus involves the conversion of the RNA genome into a DNA copy, a process referred to as reverse transcription, and its subsequent stable integration into host chromosomal DNA (64). In 1970, Howard Temin and David Baltimore substantiated the provirus hypothesis through their codiscovery of the reverse transcriptase enzyme (65, 66), a landmark achievement for which they shared the Nobel Prize in 1975 (Fig. 1). Later findings further showed that a viral-encoded integrase enzyme catalyzes integration of the viral DNA copy into cellular chromosomal DNA. This integrated viral genome, termed the provirus, allows for stable viral gene expression and viral progeny production in infected cells (57).

The unique feature of a provirus in the life cycle of RNA tumor viruses, now called retroviruses, prompted Temin to postulate that cancer may be caused by proviral-mediated implantation of oncogenes into host cells. However, different camps disagreed about whether oncogenes had a viral or cellular origin. Based on the detection of retroviral sequences in the genomes of most mammalian cells, Robert Huebner and George Todaro hypothesized that oncogenes have a viral origin and that carcinogens, irradiation, and the normal aging process cause cancer by activating these normally silent viral genes (67). This theory, named the oncogene hypothesis, represented the prevailing view of the time. By contrast, the established dispensability of the *src* gene for RSV replication urged Michael Bishop and Harold Varmus (Fig. 2) to take the minority view that oncogenes have a cellular origin and that carcinogenic events activate cellular genes to promote cancer. The latter hypothesis interestingly predicted that the reverse transcriptase–dependent life cycle of a retrovirus like RSV permits the viral genome to capture a cellular oncogene through an accident of nature.

In their seminal article published in 1976, Bishop and Varmus proved their hypothesis correct (68). To achieve this goal, they isolated a *src*-specific nucleic acid probe by annealing cDNA copies of the transformation-competent RSV genome that contained the *src* gene to genomic RNAs of a transformation-defective RSV that lacked the *src* gene, subjecting the reaction to hydroxyapatite chromatography, and specifically recovering the nonhybridized single-strand *src* cDNA sequences. Significantly, this *src* cDNA was found to hybridize with high stringency to the DNA of normal chicken cells but with somewhat lower stringency to the DNA of other avian species. This evolutionary conservation of *src* sequences provided strong evidence that *src* was indeed a cellular gene acquired by RSV from the chicken genome, rather than being a viral gene due to the highly species-specific nature of endogenous retroviruses. This finding immediately suggested to Bishop and Varmus that retroviral capture of a normal cellular gene alone would be insufficient to create an oncogene and that the cellular gene, designated a proto-oncogene, must sustain a mutation to cause cancer. This hypothesis had far-reaching implications by predicting that all cancers, not only those associated with tumor viruses, were triggered by mutagenic events that activate cellular proto-oncogenes. In 1982 and 1983, Robert Weinberg, Geoffrey Cooper, Mariano Barbacid, and colleagues substantiated this concept by showing that, compared with the *ras* proto-oncogene present in normal cells, *ras* oncogenes present in human bladder carcinoma cell lines, mouse sarcoma viruses, and rat mammary carcinomas contained a mutation crucial for inducing cellular transformation (69–71). To date, more than 70 cellular proto-oncogenes have been identified through studies of oncogenic retroviruses, and nearly all of these genes code for key cell signaling proteins involved in the control of cellular proliferation and apoptosis (57). Other studies established that retroviruses lacking viral oncogenes, such as the avian and murine leukemia viruses, induce disease by a mechanism called insertional mutagenesis. In this case, the provirus may by chance integrate near a cellular proto-oncogene and modify its expression (57). Table 2 lists examples of cellular proto-oncogenes discovered through gene capture or insertional mutagenesis by a tumorigenic retrovirus and that have been found mutated in some human cancers without a viral etiology. In 1989, Bishop and Varmus were awarded the Nobel Prize for their revolutionary discovery of the oncogene and its central role in cancer development.

DNA tumor viruses: discovery of the p53 tumor suppressor. Tumor viruses are classified in two general groups based on whether an RNA or DNA genome is packaged into the infectious viral particle. Clearly, the discovery that the oncogenic properties of RNA tumor viruses (i.e., retroviruses) stem from their capacity to capture and alter important cellular growth–regulatory genes represented a landmark achievement in cancer research. It soon became evident, however, that the oncogenes of DNA tumor viruses (e.g., SV40, mouse polyoma virus, adenovirus, and papillomavirus) differed strikingly from those of RNA tumor viruses by having both a viral origin and an essential role in viral replication. In addition, because the oncogenes of DNA tumor viruses lacked

Table 2. Examples of cellular proto-oncogenes discovered through either gene capture or insertional mutagenesis by a tumorigenic retrovirus and that have been found mutated in some human cancers

General class	Oncogene	Virus name	Protein product
Growth factor	*sis*	Simian sarcoma virus	Platelet-derived growth factor
Receptor protein tyrosine kinase	*erbB*	Avian erythroblastosis virus, Rous-associated virus 1	Epidermal growth factor receptor
	fms	McDonough feline sarcoma virus, Friend murine leukemia virus	Colony-stimulating factor receptor
	kit	Hardy-Zuckerman-4 feline sarcoma virus	Stem cell factor receptor
Nonreceptor protein tyrosine kinase	*abl*	Abelson murine leukemia virus	Tyrosine kinase
	src	Rous sarcoma virus	Tyrosine kinase
Serine/threonine protein kinase	*raf*	Murine sarcoma virus 3611	Serine/threonine kinase
	akt	AKT8 Murine leukemia virus	Serine/threonine kinase
G protein	*H-ras*	Harvey murine sarcoma virus	GDP/GTP binding
	K-ras	Kirsten murine sarcoma virus, Friend murine leukemia virus	GDP/GTP binding
Transcription factor	*erbA*	Avian erythroblastosis virus	Transcription factor (thyroid hormone receptor)
	ets	Avian myeloblastosis virus E26, Moloney murine leukemia virus	Transcription factor
	myc	MC29 myelocytoma virus, Rous associated virus 1, Moloney murine leukemia virus	Transcription factor
	rel	Reticuloendotheliosis virus	Transcription factor (NFκB family)

NOTE: Adapted from J. Butel (57) and R. Weinberg (113).

any recognizable sequence similarities to cellular genes, it remained a mystery how the products of these viral genes were able to transform cells.

The first insight into this process came from studies reported by David Lane, Arnold Levine, and colleagues in 1979 (refs. 72, 73; Fig. 2). More than 15 years earlier, Eddy and Hilleman reported that SV40 elicits tumors in experimentally infected hamsters, and several groups later showed that both the initiation and maintenance of the transformed state in SV40-infected cells depended on the expression of the viral large tumor (T) antigen, the major oncogenic determinant of SV40 (74–76). Lane and Levine used antibodies specifically reactive to SV40 large T antigen to show that immunoprecipitation from transformed cells resulted in the recovery of not only SV40 large T antigen itself but also a cellular protein having an approximate molecular weight of 53 kDa. Based on its size, this cellular protein became known as p53. Although this finding was significant in providing the first evidence that the products of DNA tumor virus oncogenes function through physical interactions with cellular proteins, a decade of additional research was required before the enormous effect of the p53 discovery would be fully comprehended.

Within a few years of the discovery of p53, the gene was cloned from neoplastic rodent and human cells and shown to have weak oncogenic activity when expressed in normal rodent cells, leading to the belief that *p53* was a cellular oncogene (77). It would not be recognized for several years, however, that these *p53* genes differed from those present in normal cells by carrying important gain-of-function mutations. This possibility first came to light in 1989 when Bert Vogelstein and colleagues reported a common loss-of-heterozygosity at the *p53* locus in human colorectal cancers (78), suggesting that *p53* was actually a tumor suppressor gene rather than an oncogene. This conclusion was rapidly verified by sequence analyses that detected frequent *p53* mutations in cancer cell lines and primary cancers (77, 79). In fact, *p53* is mutated or lost in ~50% of all human cancer cases worldwide, representing the most commonly mutated gene in human tumors. This observation, together with the extremely high spontaneous tumor incidence of *p53* knockout mice and the elevated cancer predisposition of humans with the inherited Li-Fraumeni syndrome caused by germ line *p53* mutations (80–82), provided overwhelming evidence that *p53* is a very important tumor suppressor gene. A wide variety of cellular stress stimuli, including DNA damage, aberrant proliferative signaling, and hypoxia, induce the accumulation and activation of p53, which is a sequence-specific transcription factor that also binds to and regulates the activity of several important cellular factors (77). Through these functions, p53 controls cell cycle progression, senescence, apoptosis, and DNA repair and, in so doing, prevents tumor formation by reducing the accumulation of genetic lesions, supporting the designation of p53 as the "guardian of the genome." The central importance of p53 is further underscored by the fact that, in addition to SV40 large T antigen, oncoproteins encoded by other DNA tumor viruses, such as HPV and human adenovirus, similarly evolved to bind and inactivate p53 in cells (ref. 57; Table 3). As it turns out, infections by DNA tumor viruses often trigger a p53-mediated host-cell antiviral response, which represents an attempt by the host to block virus production by preventing viral DNA synthesis and promoting apoptosis. Such viruses encode functions (i.e., oncoproteins) that counteract this cellular antiviral response by binding to and inactivating p53, allowing time for virus replication to occur. Thus, the biology of a DNA tumor virus permitted Lane and Levine to make the landmark discovery of p53 (Fig. 1).

DNA tumor viruses: discovery of functions for the retinoblastoma tumor suppressor. In 1971, Alfred Knudson reported the identification of inherited and sporadic forms of the childhood tumor retinoblastoma (Rb; ref. 83). He found that infants with the inherited disease developed several independent tumors affecting both eyes at a mean age of 14 months, whereas children with the sporadic disease instead developed a single tumor only after several years of age. Moreover, infants with the inherited disease had an approximately 30,000-fold higher chance of developing Rb than did children with the sporadic disease. Based on this information, Knudson hypothesized that both inherited and sporadic Rb susceptibility was defined by a single recessive trait and that infants with the inherited form were born with one defective and one normal allele whereas infants with the sporadic form were born with two normal alleles. Consequently, infants with the inherited disease developed tumors much more rapidly and frequently than did normal infants because rare mutation of only one instead of two tumor susceptibility alleles was required to produce disease. Knudson's hypothesis eventually proved correct, thereby providing the first evidence for the existence of cellular genes, now known as tumor suppressor genes, which in contrast to proto-oncogenes function to prevent rather than to promote cancer.

In 1986 and 1987, the *Rb* tumor suppressor gene was identified and cloned (84, 85), prompting cancer virologists to investigate whether the product of this first known tumor suppressor gene might form a complex with oncoproteins encoded by DNA tumor viruses. In 1988, Ed Harlow, David Livingston (Fig. 2), and colleagues succeeded in this endeavor by demonstrating the recovery of the cellular Rb protein in immunoprecipitates of the adenovirus E1A or the SV40 large T-antigen oncoprotein from transformed cells (86, 87). As *p53* was not recognized as a tumor suppressor gene until 1989, the discovery by Harlow and Livingston represented a milestone in tumor virology by providing the first evidence that viruses promote cancer not only by activating the products of cellular proto-oncogenes but also by inactivating the products of cellular tumor suppressor genes. Shortly thereafter, a common theme for DNA tumor viruses emerged when oncoproteins encoded by SV40, adenovirus, and HPV were found to share similar capacities for inactivating both the Rb and p53 tumor suppressors (ref. 57; Table 3).

Also significant is the fact that subsequent studies of the interactions of the human adenovirus E1A and the SV40 large T-antigen with Rb proved instrumental in deciphering the function of this cellular tumor suppressor (88). In fact, the first step in this effort had occurred earlier, prior to the identification of the *Rb* gene, when Joe Nevins (Fig. 2) and colleagues in 1986 identified the cellular transcription factor E2F (89), which was named for its ability to mediate E1A-induced transcriptional activation of the adenoviral E2 transcription unit in infected cells. Within a few years, the relationship between Rb and E2F became evident with the demonstration that a hypophosphorylated form of Rb negatively regulates G_1 to S phase progression through the cell cycle by binding to and blocking the activity of E2F, which by now was known to function in transcriptional activation of most genes involved in cellular DNA replication during S phase. It was additionally shown that the ability of cells to progress through early G_1 phase to S phase of the cell cycle depends on the accumulation of G_1 cyclin–dependent kinases, which directly hyperphosphorylate and inactivate Rb and, in so doing, release active E2F from inactive Rb/E2F complexes. Naturally, the effects of adenovirus E1A and SV40 large T antigen on the Rb/E2F pathway were examined, and the results indicated that the viral oncoproteins short-circuit the Rb/E2F pathway by specifically binding to and inactivating the hypophosphorylated form of Rb, leading to aberrant release of free active E2F and thereby unscheduled cellular proliferation of quiescent cells. The fact that the Rb/E2F pathway is dysregulated in most, if not all, human tumors illustrates the wide significance of these discoveries (90).

In summary, studies of DNA tumor viruses provided seminal contributions to our understanding of both Rb and p53, two of the most important tumor suppressor proteins in cells. The work also revealed that unscheduled cellular proliferation caused by Rb inactivation alone triggers apoptosis in cells and that p53 inactivation counteracts this adverse effect (91), explaining the frequent need for DNA tumor viruses and cancer cells to eliminate both Rb and p53 functions in cells. Thus, studies of tumor viruses played a pivotal role in demonstrating that perturbations of both proto-oncogenes and tumor suppressor genes drive the malignant growth of cancer cells.

The 1980s to the Present: Additional Human Tumor Viruses

The most recent milestones in tumor virology have come from the identification of additional human tumor viruses: human T-cell leukemia virus type 1 (HTLV-1), hepatitis C virus (HCV), and Kaposi's sarcoma virus.

HTLV-1: the first tumorigenic human retrovirus. In 1977, Kiyoshi Takatsuki and colleagues discovered a variable T-cell leukemia in Japanese adults with a unique set of properties that

Table 3. DNA virus and complex retrovirus oncoproteins and cellular protein interactions

Virus	Viral oncoprotein	Cellular targets*
SV40	Large T antigen	p53, Rb
	Small t-antigen	PP2A
Polyoma	Large T-antigen	Rb
	Middle T-antigen	Src, PI3K, PLC-γ, Shc
	Small t-antigen	PP2A
Adeno	E1A	Rb
	E1B-55K	p53
Adeno type 9	E4-ORF1	Dlg1, PATJ, ZO-2, MAGI-1, MUPP1
HPV	E6	p53, Dlg1, Scribble, PATJ, MAGI-1, MUPP1
	E7	Rb
HTLV-1	Tax	NFκB, p300/CBP, Dlg1, Scribble
BPV	E5	PDGFβ receptor
EBV	LMP1	TRAFs

NOTE: Adapted from J. Butel (57).
Abbreviations: Adeno, adenovirus; BPV, bovine papillomavirus; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol-3 kinase; Polyoma, mouse polyomavirus; PLC-γ, phospholipase C-γ; PP2A, protein phosphatase 2A; TRAF, tumor necrosis factor receptor–associated factor.
*Additional cellular proteins are reported to interact with some of the viral oncoproteins. This list is representative, not exhaustive.

warranted the classification of the disease as a single syndrome called adult T-cell leukemia (ATL; ref. 92). Reminiscent of Burkitt's lymphoma, ATL showed a distinct geographic distribution in Japan, with most cases clustered on the southern islands of Kyushu and Okinawa and the northern island of Hokkaido and with only sporadic cases found in remote coastal villages along the largest island of Honshu. These observations suggested the possibility of an infectious etiologic agent for ATL.

In the 1970s, decades of attempts to identify a human retrovirus had failed, despite the successful isolation of many animal retroviruses. Nonetheless, Robert Gallo remained steadfast in this extremely unpopular endeavor and, because animal retroviruses most often induced leukemia, he focused his quest on human retroviruses in human leukemia. In 1980, this effort by Gallo and colleagues was finally rewarded when they detected retroviral reverse transcriptase activity and visualized retroviral particles in cultured human T-cell lymphoma cells (93). The agent proved to be immunologically distinct from other known viruses. Thus, Gallo had discovered the first human retrovirus, which was named HTLV-1. At the time, however, it was unclear whether HTLV-1 actually played a role in promoting leukemia. In 1981, Yorio Hinuma, Kinya Nagata, Isao Miyoshi, and colleagues addressed this point when they reported similar visualization of retroviral particles produced by a leukemia cell line derived from patients with ATL (94). This virus turned out to be identical to HTLV-1. These investigators linked HTLV-1 to ATL by showing that patients with ATL but not control individuals produced antibodies that specifically recognized antigens expressed in HTLV-1–infected human T-cells. These landmark findings suggested a causal role for HTLV-1 in ATL (Table 1).

Over the next few years, evidence supporting the association of HTLV-1 with ATL rapidly accumulated (27). First, the geographic distribution of ATL in Japan matched that of HTLV-1 infections, and new cases of HTLV-I infection discovered along central African coastal regions, and less frequently in the Caribbean basin, Taiwan, and Papua New Guinea, were also linked to ATL. Second, virtually every ATL patient had experienced an HTLV-1 infection. Third, all leukemic cells cultured from patients with ATL contained HTLV-1 proviral DNA. Fourth, HTLV-1 infection of normal human T cells induced transformation as evidenced by cellular immortalization. Fifth, bovine leukemia virus and simian T-cell leukemia virus 1, retroviruses closely related to HTLV-1, caused leukemia in their respective animal hosts. Such results from epidemiologic and molecular studies overwhelmingly implicated HTLV-1 as the etiologic agent of ATL. Underscoring the significance of this discovery, an estimated 10 to 20 million people worldwide are infected with HTLV-1, and the prognosis for patients with ATL remains poor (95). Interestingly, HTLV-1 still represents the only known human retrovirus linked directly to a specific human malignancy.

Subsequent findings unexpectedly indicated that, contrary to mechanisms typical of animal retroviruses, HTLV-1 does not cause cancer by insertional mutagenesis or by capturing and activating cellular proto-oncogenes. Rather, more reminiscent of a DNA tumor virus, the major oncogenic determinant of HTLV-1 is the viral *Tax* gene that encodes a protein essential for viral replication (95). The transforming properties of viral Tax seem to involve a transcriptional activating function, perturbations of various cell growth–regulatory factors, and interference with the cellular DNA repair apparatus. An additional striking feature of HTLV-1–induced disease is that, despite the typical occurrence of HTLV-1 infections at an early age, <5% of such individuals develop ATL and those after an extraordinarily prolonged latent period ranging from 20 to 30 years. This observation illustrates another general concept of tumor virology in which many years generally pass between initial infection and tumor appearance, and most infected individuals do not develop cancer. In the case of HTLV-1 infection, it has been speculated that, during the long latent period of HTLV-1 infection, rare genetic and epigenetic changes slowly accumulate in the context of genetic susceptibility factors and Tax-mediated dysregulated cell growth to promote leukemia in a small percentage of infected individuals.

HCV: the second human virus linked to HCC. With the discovery of HBV and the development of highly sensitive assays to detect HBV antigens, it became possible to identify virtually all patients infected by HBV. In the early 1970s, results obtained with these assays unexpectedly showed that most patients with transfusion-associated serum hepatitis were not infected with HBV (96). Furthermore, hepatitis A virus, a newly identified etiologic agent for hepatitis in people, likewise was not responsible for cases of post-transfusion hepatitis, now referred to as non–A, non–B hepatitis. During the next 15 years, much was learned about the etiologic agent, the disease, and the epidemiology of non–A, non–B hepatitis. Although the acute disease was often relatively mild, individuals ultimately developed chronic infections that could lead to serious chronic liver disease and cirrhosis. In addition, the disease could be transmitted to chimpanzees. Using this animal model, researchers showed that the etiologic agent was a small, enveloped virus, and efforts were initiated to identify the virus.

Advanced molecular biological techniques developed in the 1980s would play a major role in the identification of the non–A, non–B hepatitis virus. To achieve this goal, Michael Houghton and a group of scientists at the Chiron Corporation generated a lambda phage cDNA expression library from nucleic acid extracted from plasma of a chronically infected chimpanzee. This library was screened with serum from a chronic non–A, non–B hepatitis patient presumed to have antibodies to the virus. In 1989, Houghton and colleagues reported that this approach led to the identification of a lambda phage clone that expressed an antigen encoded by a previously unknown RNA virus, which was named HCV (97). Surprisingly, like yellow fever virus, HCV was found to be a flavivirus, a virus family not thought to contain oncogenic members. Using the first serologic test for HCV, which was developed from the viral expression product of this phage clone, Houghton confirmed that HCV was indeed the etiologic agent for post-transfusion non–A, non–B hepatitis (98, 99).

Given the established link between HBV-induced chronic liver disease and liver cancer, Houghton and colleagues reported a similar association of chronic HCV infection with HCC, amazingly in the same year that HCV was identified (ref. 100; Table 1). It is now known that HCV is second only to HBV in causing chronic hepatitis, liver cirrhosis, and HCC worldwide (101). Among patients infected with HCV, it is almost exclusively those with cirrhosis who develop HCC, revealing a major risk factor for malignant progression. The impact of this observation can be realized when one considers that chronic HCV infections affect >170 million individuals worldwide and that an estimated 20% of these people have or will develop cirrhosis. For the latter patients, the annual risk for developing HCC is 1% to 4%, with patients from Japan having an even higher risk. Consequently, similar to HBV infection, chronic inflammation and cirrhosis are believed to play key roles in

promoting HCV-associated HCC, although the underlying mechanisms of this process are not yet understood.

Kaposi's sarcoma virus: a second tumorigenic human herpesvirus. First described in Eastern Europe in 1872, Kaposi's sarcoma is an endemic tumor of the Mediterranean basin and Africa (102). The classic endemic form of the disease primarily affects elderly males, in which tumors are generally restricted to the skin and are rarely life-threatening. With the advent of the HIV epidemic in the 1980s, however, it soon became evident that acquired immune deficiency syndrome (AIDS) patients commonly developed a more aggressive form of Kaposi's sarcoma, manifesting not only as widespread and often disfiguring dermal lesions but also with frequent involvement of extracutaneous sites, typically the lungs and gastrointestinal tract, which result in serious complications. The observation that untreated AIDS patients exhibit a 20,000-fold higher risk for developing Kaposi's sarcoma than do the general population prompted large-scale epidemiologic studies that suggested an infectious etiology for the tumor. Although HIV was an obvious suspect, a variety of epidemiologic and experimental evidence ruled out this possibility, thereby initiating the search for an unknown sexually transmitted infectious agent playing a causal role in Kaposi's sarcoma.

As with the search for HCV, a modern molecular biological approach would likewise play a central role in the identification of Kaposi's sarcoma virus. In 1993, representational difference analysis was first described. With this method, DNA sequences present in diseased tissue but absent from healthy tissue of the same patient could be specifically enriched and thereby identified through successive rounds of PCR amplification and subtractive hybridization of fragmented DNA from each tissue (103). In a seminal article published in 1994, Yuan Chang, Patrick Moore, and colleagues reported the use of this powerful new technique to identify several novel DNA fragments distantly homologous to the herpesvirus EBV in 90% of Kaposi's sarcoma tissues from AIDS patients that were not found in tissues from non–AIDS patients (104). This new virus was called Kaposi's sarcoma herpesvirus (KSHV) or human herpesvirus type 8. In short order, molecular clones of the entire KSHV genome were isolated and sequenced, facilitating the development of both PCR tests and serologic assays that permitted the detection of the virus in clinical samples (102).

These tools were used to show that Kaposi's sarcomas, including both AIDS-related and AIDS-unrelated forms of the disease, invariably contain KSHV DNA and that the prevalence of KSHV infection is high in groups that commonly develop the tumor, but low in those that rarely do. Furthermore, in patients with AIDS, KSHV infection precedes the onset of Kaposi's sarcoma and results in an elevated risk for tumor development. Evidence additionally indicated that KSHV is sexually transmitted among male homosexuals in the United States, consistent with the epidemiologic data. These collective data taken from the study of thousands of subjects strongly indicate a causal role for KSHV in the development of Kaposi's sarcoma (ref. 102; Table 1). Nonetheless, KSHV infection is clearly not sufficient to produce the disease. For example, 2% to 7% of the population from the United States scored positive for KSHV in serologic assays yet displayed no measurable increased risk for Kaposi's sarcoma, implying a role for cofactors in tumor development. Whereas the cofactors involved in classic Kaposi's sarcoma have not been determined, HIV infection is an established cofactor in the AIDS-related disease. The mechanisms by which HIV contributes to Kaposi's sarcoma development are not understood, with *in vitro* data suggesting that HIV augments KSHV replication and *in vivo* data suggesting that HIV promotes tumor cell survival and growth by up-regulating cytokine production. Additionally, HIV is considered a contributing factor in all types of AIDS-related malignancies because of its immunosuppressive effects on the host immune system. Clearly, more work is needed to determine exactly how HIV infection contributes to Kaposi's sarcoma.

Summary and Conclusions

For 40 years following the discovery of the first tumor virus by Peyton Rous in 1911, viruses were viewed as peculiar infectious agents capable of inducing cancer in animals but having no relevance to humans. However, by the end of the 20th century, compelling evidence had accumulated that six different human viruses, including EBV, HBV, HPV, HTLV-1, HCV, and KSHV, were bona fide etiologic agents of human cancer and caused 15% to 20% of all human tumors worldwide (refs. 57, 105; Table 1). During this time, tumor viruses also proved their worth as powerful tools for revealing fundamental molecular events that trigger the development of all human cancers, regardless of etiology. Both RNA and DNA tumor viruses contributed distinct insights into this disease process by revealing central roles for cellular oncogene activation and tumor suppressor gene inactivation, respectively. Most known cellular oncogenes have been identified through studies of RNA tumor viruses, whereas identification of the p53 tumor suppressor and many functions of the Rb tumor suppressor were gleaned through studies of DNA tumor viruses. Without these seminal contributions of tumor virology, it is difficult to envision that our understanding of cancer would be revealed at the depth that we appreciate today. In the 21st century, it is expected that tumor viruses will continue to serve as tools for discovery. As one example, findings indicate that oncoproteins encoded by adenovirus, HPV, and HTLV-1 commonly target and block the function of cellular factors required for the establishment of proper cell polarity (Dlg1, Scribble, PATJ, ZO-2; Table 3), a property lost in nearly all epithelial-derived cancer cells (106). Thus, given accumulating evidence suggesting that loss of cell polarity directly contributes to malignant progression (107), studies of tumor viruses may aid in revealing how this common defect contributes to the development of many human cancers.

We anticipate that the list of human tumor viruses will grow considerably longer in the future. This will include recognition of known viruses having a role in human cancer, as well as identification of new candidate cancer viruses through the use of modern molecular technology. In particular, there is emerging interest in the polyomaviruses as possible human carcinogens (refs. 108, 109; Table 1). SV40, which naturally infects the rhesus monkey, was inadvertently introduced into the human population as a contaminant of early poliovirus vaccines, whereas the BK and JC polyomaviruses are natural human pathogens associated with disease processes in the urinary tract or brain, respectively. DNA sequences of these three polyomaviruses, which are tumorigenic under experimental conditions, have been detected in mesothelioma, osteosarcoma, non–Hodgkin lymphoma, brain tumors, and prostate cancer. In addition, an integrated form of a new polyomavirus, Merkel cell polyomavirus, was recently observed in Merkel cell carcinoma, a rare but aggressive human skin cancer of neuroendocrine origin (110).

Several other candidate human tumor viruses have also been documented (105). Although early investigations failed to associate

adenovirus with human cancer, a recent study found adenoviral DNA in pediatric brain tumors. Human endogenous retroviruses, which are sequences resembling infectious retroviruses in the human genome, have been shown to display increased viral mRNA and protein expression as well as retrovirus-like particle production in seminomas, breast cancer, myeloproliferative disease, ovarian cancer, melanoma, and prostate cancer. Likewise, particles of human mammary tumor virus, a retrovirus similar to mouse mammary tumor virus, were detected in primary human breast cancer cells. Finally, protein expression by a newly discovered xenotropic murine leukemia virus–related virus was found in the stroma of human prostate tumors, and DNA sequences of torque teno virus were observed in myeloma and cancers of the gastrointestinal tract, lung, and breast. As the mere presence of viral sequences is insufficient to conclude that a virus contributes to tumor formation, the causal role of these viruses in human cancer remains unresolved. It is challenging and usually requires years of research to achieve wide acceptance of the viral causation for a given human cancer (31, 51, 57, 108). The challenge is even greater if only a subset of the cancers in question have a viral etiology. Nonetheless, we predict that, in the 21st century, viruses will be associated with substantially more than the current estimate of 15% to 20% of all human cancers.

Many tumor viruses play an essential role in both the early initiation and subsequent progression of cancer, as evidenced by a requirement for viral oncogene functions at all stages of the neoplasm. Nonetheless, in the future, more consideration must be given to the possibility that viral functions required for early tumor initiation may occasionally become dispensable upon the selection of more growth-autonomous tumor cells, thereby permitting the loss of viral sequences during progression to late stage tumors. Although this so-called "hit-and-run" mechanism for carcinogenesis is intrinsically difficult to prove, the recent demonstration that certain viral oncoproteins (e.g., HPV E7 and HTLV-1 Tax) induce mutations and reduce genomic stability by impairing the cellular DNA repair system support this concept (105). Such effects could conceivably increase the rate at which mutations accumulate in growth-regulatory genes, thereby promoting tumor formation independent of a need for the continued presence of viral sequences. Another scenario that must also be considered is that viral sequences and viral gene expression are maintained in only a small fraction of cancer cells, which secrete factors that promote abnormal proliferation of surrounding uninfected cells in a tumor. These proposed mechanisms challenge the widely held belief that viral sequences will be found in every cell if the virus is etiologically important in the genesis of the tumor.

Prophylactic vaccines are the most effective approach in the prevention of viral disease because they induce antibodies that efficiently neutralize viruses prior to the establishment of an acute or chronic infection. Prophylactic vaccination also offers the potential to prevent cancers having a viral etiology, as has been shown for both HBV-associated and HPV-associated diseases. The latter successes warrant that a major emphasis be placed on the development of new vaccines against other human tumor viruses, given that such efforts represent an opportunity to decrease the incidence of cancer worldwide. It is possible that new targets will be identified from viral pathogenesis studies to prompt the development of antiviral drugs to treat chronic infections by human tumor viruses. If such infections could be controlled or eliminated, the subsequent development of virus-induced cancer in the host would be reduced.

Viruses have also been used as therapeutic tools to augment conventional cancer chemotherapy or immunotherapy. One approach has been the use of oncolytic viruses capable of killing cancer cells but not normal cells. A virus that has already shown some therapeutic promise is the oncolytic adenovirus mutant Onyx-015 (111). The ability of the adenovirus to replicate lytically in normal cells depends on the viral *E1B* gene and its ability to promote late viral gene expression required for progeny virus production and to shut off host cell gene expression. Onyx-015 carries an *E1B* gene mutation that prevents lytic replication in normal cells but permits selective lytic replication in cancer cells, which in general, conveniently complement the Onyx-015 *E1B* replication deficiency. In a phase II trial for patients with head and neck tumors, intratumoral injection of Onyx-015 combined with systemic chemotherapy caused 19 out of 30 tumors to decrease in size by 50% or more and 8 tumors to completely regress. Other types of oncolytic viruses in development include vaccinia, herpes simplex virus type 1, reovirus, and Newcastle disease virus (112). Viral vectors are likewise being used to deliver therapeutic genes designed to kill or block the growth of tumor cells or boost the immune system to target and destroy such cells. Although oncolytic viruses and viral vectors are at early stages of development and many limitations must still be solved, these approaches are expected to yield significant therapeutic benefits in the treatment and prevention of human cancers in the 21st century.

In sum, tumor virology has played a central role in cancer research since the middle of the 20th century. Viruses have been identified as infectious causes of specific human cancers and have advanced our general understanding of the molecular basis of carcinogenesis. We predict that tumor virology will continue to be an invaluable partner in cancer research efforts in the 21st century.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 8/25/2008; accepted 8/25/2008.

References

1. Mackay J, Jemal A, Lee NC, Parkin DM. The history of cancer. The Cancer Atlas, 1st ed. American Cancer Society; 2006.
2. Diamandopoulos GT. Cancer: an historical perspective. Anticancer Res 1996;16:1595–602.
3. Gallucci BB. Selected concepts of cancer as a disease: from the Greeks to 1900. Oncol Nurs Forum 1985;12: 67–71.
4. Kardinal CG, Yarbro JW. A conceptual history of cancer. Semin Oncol 1979;6:396–408.
5. Levine AJ. The Origins of Virology, In: Knipe DM, Howley PM, editors. Fields virology, 4th ed, vol. 1. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 3–18.
6. Ciuffo G. Innesto positivo con filtrato di verruca volgare. Giorn Ital Mal Venereol 1907;48:12–7.
7. Ellermann V, Bang O. Experimentelle leukamie bei huhnern. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg 1908;46:595–7.
8. Epstein MA. Historical background. Philos Trans R Soc Lond B Biol Sci 2001;356:413–20.
9. Rous P. A sarcoma of the fowl transmissible by an

agent separable from the tumor cells. J Exp Med 1911;13:397–9.
10. Rous P. A transmissable avian neoplasm. J Exp Med 1910;12:696–705.
11. Vogt PK. Peyton Rous: homage and appraisal. FASEB J 1996;10:1559–62.
12. Shope RE, Hurst EW. Infectious papillomatosis of rabbits; with a note on the histopathology. J Exp Med 1933;58:607–24.
13. Rous P, Beard JW. The progression to carcinoma of virus-induced rabbit papillomas (Shope). J Exp Med 1935;62:523–48.
14. Bittner JJ. Some possible effects of nursing on the mammary tumor incidence in mice. Science 1936;84:162–9.
15. Gross L. "Spontaneous" leukemia developing in C3H mice following inoculation in infancy, with AK-leukemic extracts, or AK-embrvos. Proc Soc Exp Biol Med 1951;76:27–32.
16. Gross L. A filterable agent, recovered from Ak leukemic extracts, causing salivary gland carcinomas in C3H mice. Proc Soc Exp Biol Med 1953;83:414–21.
17. Friend C. Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. J Exp Med 1957;105:307–18.
18. Graffi A. Chloroleukemia of mice. Ann N Y Acad Sci 1957;68:540–58.
19. Moloney JB. Biological studies on a lymphoid-leukemia virus extracted from sarcoma 37. I. Origin and introductory investigations. J Natl Cancer Inst 1960;24:933–51.
20. Hilleman MR, Werner JH. Recovery of new agent from patients with acute respiratory illness. Proc Soc Exp Biol Med 1954;85:183–8.
21. Rowe WP, Huebner RJ, Gilmore LK, Parrott RH, Ward TG. Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc Soc Exp Biol Med 1953;84:570–3.
22. Trentin JJ, Yabe Y, Taylor G. The quest for human cancer viruses. Science 1962;137:835–41.
23. Sweet BH, Hilleman MR. The vacuolating virus, SV40. Proc Soc Exp Biol Med 1960;105:420–7.
24. Eddy BE, Borman GS, Grubbs GE, Young RD. Identification of the oncogenic substance in rhesus monkey kidney cell culture as simian virus 40. Virology 1962;17:65–75.
25. Girardi AJ, Sweet BH, Slotnick VB, Hilleman MR. Development of tumors in hamsters inoculated in the neonatal period with vacuolating virus, SV-40. Proc Soc Exp Biol Med 1962;109:649–60.
26. Burkitt D. A children's cancer dependent on climatic factors. Nature 1962;194:232–4.
27. Levine AJ. Viruses. New York: Scientific American Library; 1991.
28. Epstein MA, Henle G, Achong BG, Barr YM. Morphological and biological studies on a virus in cultured lymphoblasts from Burkitt's lymphoma. J Exp Med 1965;121:761–70.
29. Henle G, Henle W. Immunofluorescence in cells derived from Burkitt's lymphoma. J Bacteriol 1966;91:1248–56.
30. Liao JB. Viruses and human cancer. Yale J Biol Med 2006;79:115–22.
31. Pagano JS, Blaser M, Buendia MA, et al. Infectious agents and cancer: criteria for a causal relation. Semin Cancer Biol 2004;14:453–71.
32. Hollinger FB, Liang TJ. Hepatitis B virus. In: Knipe DM, Howley PM, editors. Fields virology, 4th ed, vol. 2. Philadelphia: Lipppincott Williams & Wilkins; 2001. p. 2971–3036.
33. Blumberg BS, Alter HJ, Visnich S. A "new" antigen in leukemia sera. JAMA 1965;191:541–6.
34. Blumberg BS, Gerstley BJ, Hungerford DA, London WT, Sutnick AI. A serum antigen (Australia antigen) in Down's syndrome, leukemia, and hepatitis. Ann Intern Med 1967;66:924–31.
35. Okochi K, Murakami S. Observations on Australia antigen in Japanese. Vox Sang 1968;15:374–85.
36. Prince AM. An antigen detected in the blood during the incubation period of serum hepatitis. Proc Natl Acad Sci U S A 1968;60:814–21.
37. Ganem D, Schneider R. Hepadnaviridae: the viruses and their replication. In: Knipe DM, Howley PM, editor. Fields virology, 4th ed, vol. 2. Philadelphia: Lipppincott Williams & Wilkins; 2001. p. 2923–70.
38. Blumberg BS, Larouze B, London WT, et al. The relation of infection with the hepatitis B agent to primary hepatic carcinoma. Am J Pathol 1975;81:669–82.
39. Beasley RP, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22,707 men in Taiwan. Lancet 1981;2:1129–33.
40. Buynak EB, Roehm RR, Tytell AA, Bertland AU II, Lampson GP, Hilleman MR. Vaccine against human hepatitis B. JAMA 1976;235:2832–4.
41. Hilleman MR. Critical overview and outlook: pathogenesis, prevention, and treatment of hepatitis and hepatocarcinoma caused by hepatitis B virus. Vaccine 2003;21:4626–49.
42. Chang MH, Chen CJ, Lai MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. N Engl J Med 1997;336:1855–9.
43. zur Hausen H. Papillomavirus infections—a major cause of human cancers. Biochim Biophys Acta 1996;1288:F55–78.
44. Olson C, Pamukcu AM, Brobst DF, Kowalczyk T, Satter EJ, Price JM. A urinary bladder tumor induced by a bovine cutaneous papilloma agent. Cancer Res 1959;19:779–82.
45. Jablonska S, Dabrowski J, Jakubowicz K. Epidermodysplasia verruciformis as a model in studies on the role of papovaviruses in oncogenesis. Cancer Res 1972;32:583–9.
46. zur Hausen H. Condylomata acuminata and human genital cancer. Cancer Res 1976;36:794.
47. zur Hausen H, Meinhof W, Scheiber W, Bornkamm GW. Attempts to detect virus-specific DNA in human tumors. I. Nucleic acid hybridizations with complementary RNA of human wart virus. Int J Cancer 1974;13:650–6.
48. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J 1984;3:1151–7.
49. Durst M, Gissmann L, Ikenberg H, zur Hausen H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. Proc Natl Acad Sci U S A 1983;80:3812–5.
50. Frazer IH, Lowy DR, Schiller JT. Prevention of cancer through immunization: prospects and challenges for the 21st century. Eur J Immunol 2007;37 Suppl 1:S148–55.
51. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002;2:342–50.
52. Ghim SJ, Jenson AB, Schlegel R. HPV-1 L1 protein expressed in cos cells displays conformational epitopes found on intact virions. Virology 1992;190:548–52.
53. Kirnbauer R, Booy F, Cheng N, Lowy DR, Schiller JT. Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. Proc Natl Acad Sci U S A 1992;89:12180–4.
54. Rose RC, Bonnez W, Reichman RC, Garcea RL. Expression of human papillomavirus type 11 L1 protein in insect cells: *in vivo* and *in vitro* assembly of viruslike particles. J Virol 1993;67:1936–44.
55. Zhou J, Sun XY, Stenzel DJ, Frazer IH. Expression of vaccinia recombinant HPV 16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles. Virology 1991;185:251–7.
56. Schiller JT, Lowy DR. Prospects for cervical cancer prevention by human papillomavirus vaccination. Cancer Res 2006;66:10229–32.
57. Butel JS. Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. Carcinogenesis 2000;21:405–26.
58. Keogh EV. Ectodermal lesions produced by the virus of Rous sarcoma. Br J Exp Path 1938;19:1–9.
59. Temin HM, Rubin H. Characteristics of an assay for Rous sarcoma virus and Rous sarcoma cells in tissue culture. Virology 1958;6:669–88.
60. Martin GS. The road to Src. Oncogene 2004;23:7910–7.
61. Duesberg PH, Vogt PK. Differences between the ribonucleic acids of transforming and nontransforming avian tumor viruses. Proc Natl Acad Sci U S A 1970;67:1673–80.
62. Temin HM. The effects of actinomycin D on growth of rous sarcoma virus *in vitro*. Virology 1963;20:577–82.
63. Temin HM. The participation of DNA in Rous sarcoma virus production. Virology 1964;23:486–94.
64. Temin HM. Nature of the provirus of Rous sarcoma. Nat Cancer Inst Monograph 1964;17:557–70.
65. Baltimore D. RNA-dependent DNA polymerase in virions of RNA tumour viruses. Nature 1970;226:1209–11.
66. Temin HM, Mizutani S. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature 1970;226:1211–3.
67. Huebner RJ, Todaro GJ. Oncogenes of RNA tumor viruses as determinants of cancer. Proc Natl Acad Sci U S A 1969;64:1087–94.
68. Stehelin D, Varmus HE, Bishop JM, Vogt PK. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. Nature 1976;260:170–3.
69. Parada LF, Tabin CJ, Shih C, Weinberg RA. Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus ras gene. Nature 1982;297:474–8.
70. Der CJ, Krontiris TG, Cooper GM. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. Proc Natl Acad Sci U S A 1982;79:3637–40.
71. Sukumar S, Notario V, Martin-Zanca D, Barbacid M. Induction of mammary carcinomas in rats by nitrosomethylurea involves malignant activation of H-ras-1 locus by single point mutations. Nature 1983;306:658–61.
72. Lane DP, Crawford LV. T antigen is bound to a host protein in SV40-transformed cells. Nature 1979;278:261–3.
73. Linzer DI, Levine AJ. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell 1979;17:43–52.
74. Brugge JS, Butel JS. Role of simian virus 40 gene A function in maintenance of transformation. J Virol 1975;15:619–35.
75. Martin RG, Chou JY. Simian virus 40 functions required for the establishment and maintenance of malignant transformation. J Virol 1975;15:599–612.
76. Tegtmeyer P. Function of simian virus 40 gene A in transforming infection. J Virol 1975;15:613–8.
77. Braithwaite AW, Prives CL. p53: more research and more questions. Cell Death Differ 2006;13:877–80.
78. Vogelstein B, Fearon ER, Kern SE, et al. Allelotype of colorectal carcinomas. Science 1989;244:207–11.
79. Finlay CA, Hinds PW, Levine AJ. The p53 proto-oncogene can act as a suppressor of transformation. Cell 1989;57:1083–93.
80. Donehower LA, Harvey M, Slagle BL, et al. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 1992;356:215–21.
81. Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990;250:1233–8.
82. Srivastava S, Zou ZQ, Pirollo K, Blattner W, Chang EH. Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. Nature 1990;348:747–9.
83. Knudson AG, Jr. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A 1971;68:820–3.
84. Friend SH, Bernards R, Rogelj S, et al. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 1986;323:643–6.
85. Lee WH, Bookstein R, Hong F, Young LJ, Shew JY, Lee EY. Human retinoblastoma susceptibility gene: cloning, identification, and sequence. Science 1987;235:1394–9.
86. DeCaprio JA, Ludlow JW, Figge J, et al. SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. Cell 1988;54:275–83.
87. Whyte P, Buchkovich KJ, Horowitz JM, et al. Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. Nature 1988;334:124–9.

88. Nevins J. Cell transformation by viruses, In: Knipe DM, Howley PM, editors. Fields virology, 4th ed, vol. 1. Philadelphia: Lipppincott Williams & Wilkins; 2001. p. 245–84.

89. Kovesdi I, Reichel R, Nevins JR. Identification of a cellular transcription factor involved in E1A transactivation. Cell 1986;45:219–28.

90. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell 1995;81:323–30.

91. Hickman ES, Moroni MC, Helin K. The role of p53 and pRB in apoptosis and cancer. Curr Opin Genet Dev 2002;12:60–6.

92. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. Adult T-cell leukemia: clinical and hematologic features of 16 cases. Blood 1977;50:481–92.

93. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci U S A 1980;77:7415–9.

94. Hinuma Y, Nagata K, Hanaoka M, et al. Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. Proc Natl Acad Sci U S A 1981;78:6476–80.

95. Matsuoka M, Jeang KT, Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. Nat Rev Cancer 2007;7:270–80.

96. Major ME, Rehermann B, Feinstone SM, Hepatitis C viruses. In: Knipe DM, Howley PM, editors. Fields virology, vol. 1. Philadelphia: Lippincott, Williams & Wilkins; 2001. p. 1127–62.

97. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989;244:359–62.

98. Alter HJ, Purcell RH, Shih JW, et al. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Engl J Med 1989;321:1494–500.

99. Kuo G, Choo QL, Alter HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989;244:362–364.

100. Colombo M, Kuo G, Choo QL, et al. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. Lancet 1989;2:1006–8.

101. Tan A, Yeh SH, Liu CJ, Cheung C, Chen PJ. Viral hepatocarcinogenesis: from infection to cancer. Liver Int 2008;28:175–88.

102. Ganem D. KSHV infection and the pathogenesis of Kaposi's sarcoma. Annu Rev Pathol 2006;1:273–96.

103. Lisitsyn N, Lisitsyn N, Wigler M. Cloning the differences between two complex genomes. Science 1993;259:946–51.

104. Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 1994;266:1865–9.

105. McLaughlin-Drubin ME, Munger K. Viruses associated with human cancer. Biochim Biophys Acta 2008; 1782:127–50.

106. Bilder D. Epithelial polarity and proliferation control: links from the Drosophila neoplastic tumor suppressors. Genes Dev 2004;18:1909–25.

107. Dow LE, Humbert PO. Polarity regulators and the control of epithelial architecture, cell migration, and tumorigenesis. Int Rev Cytol 2007;262:253–302.

108. Butel JS. SV40, human infections, and cancer: emerging concepts and causality considerations. In: Khalili K, Jeang KT, editors. Viral oncology: basic to clinical perspectives. J Wiley. In press 2008.

109. White MK, Khalili K. Polyomaviruses and human cancer: molecular mechanisms underlying patterns of tumorigenesis. Virology 2004;324:1–16.

110. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science 2008;319:1096–100.

111. Alemany R. Cancer selective adenoviruses. Mol Aspects Med 2007;28:42–58.

112. Cross D, Burmester JK. Gene therapy for cancer treatment: past, present and future. Clin Med Res 2006;4: 218–27.

113. Weinberg RA. The biology of cancer. New York: Garland Science; 2007.

114. Khuri FR, Nemunaitis J, Ganly I, et al. A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. Nat Med 2000;6:879–85.

115. Lamont JP, Nemunaitis J, Kuhn JA, Landers SA, McCarty TM. A prospective phase II trial of ONYX-015 adenovirus and chemotherapy in recurrent squamous cell carcinoma of the head and neck (the Baylor experience). Ann Surg Oncol 2000;7:588–92.