Chapter 2
Human Tumor Viruses: A Historical Perspective

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Abstract The fact that infectious diseases and cancer might be often associated either as the complication or cause has been discussed for decades. However, reliable epidemiological and experimental data demonstrating that numerous infectious agents can be etiological factors of human malignancies appeared only in recent years. This chapter overviews data on virus-associated malignancies and discusses potential mechanisms responsible for this association.

Keywords Epstein-Barr virus • Burkitt lymphoma • Nasopharyngeal Carcinoma • Kaposi's sarcoma • Hepatocellular Carcinoma

Introduction

That infection and cancer are so often associated either as complication or cause has been known for many years. However viruses and other infectious agents have emerged only in recent decades as etiological factors of human malignancies. Epstein-Barr virus, discovered 50 years ago (1964) and characterized early as the first human tumor virus, although its exact roles in the malignancies with which it is associated are not straightforward, continues to present the quandaries of ascertaining etiology. Helicobacter pylori was rejected as cause of gastric cancer for years before being accepted, in part because it was not a virus with the then-known mechanisms for transformation at its disposal.

Although avian and rodent viruses were accepted as causes of malignancies in these species as early as 1911, the barrier to viruses in human cancer seemed firm. Means to breach the barrier were being developed unwittingly by John Enders in the 1960s when he devised means to cultivate human cells in culture for the study of poliovirus infection. Not however until 1958 was there an opening to oncogenic studies: Jan Ponten working at the Wistar Institute in Philadelphia showed that a mammalian virus SV 40 could transform normal cells in culture into cells with

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malignant features. The virus, discovered as a contaminant from the monkey kidneys used to make poliovirus vaccine, was simian not human in origin, but the changes in phenotype that the virus could produce in human diploid fibroblasts were striking: unchecked growth of the cells with loss of contact and mitotic inhibition and stunning transformation of cellular morphology. The field of human tumor virology was launched.

Moreover SV40, a polyomavirus, could produce tumors when injected into hamsters, but not when injected into monkeys, the species from which the virus had been isolated. Nevertheless exploration of SV40 as a possible factor in brain tumors and lymphomas continued for some years, but an association with either remained uncertain. There is, however, evidence that the virus may contribute to pathogenesis of mesothelioma by causing perturbations in cell cycle. In any case SV40 did catalyze the search for a human tumor virus.

The human polyomaviruses, JCV and BKV, which were discovered later, could infect humans, but neither produced tumors. JCV infects the brain where it is the causative agent of Progressive Multifocal Leucoencephalopathy, and it could transform human glial cells in culture but does not cause brain tumors. BKV primarily infects the human urogenital track, is weakly oncogenic in cells from a variety of species of experimental animals, but does not cause tumors.

Only with the discovery of Merkel Cell Virus (MCV) in 2007 was an oncogenic polyomavirus identified (White et al. 2014). It produces a rare tumor of Merkel cells, Merkel cell carcinoma (MCC) in the skin, although intriguingly the virus could be detected in ~80 % but not all MCC. Current thinking is that MCV infection of Merkel cells drives their clonal proliferation. Search for MCV in other skin conditions continues. Intriguingly there are a growing number of new human polyomaviruses, isolated mainly from the respiratory track, but not yet coupled with disease, that should open a new frontier in tumor virology.

The most studied of infectious agents associated with generation of cancer are viruses, & discovery of new tumor viruses continues. That up to 20 % of malignancies are caused by viruses is probably an underestimate in regions of the world such as Africa and Brazil. Not only are additional viruses being identified as oncogenic, but new mechanisms are being uncovered. Examples known for years include inactivation of cellular tumor-suppressor genes, activation of cellular oncogenes and insertional mutagenesis, mostly in latent infection. More recently the importance of inflammatory reactions generated by active viral infection has been appreciated along with the expression of cytokines, both induced and virally encoded. Additionally the importance of cell type as well as viral genotype has emerged, and roles for cofactors identified.

Epstein-Barr Virus

This human herpesvirus epitomizes the levels through which a virus can be linked to malignancy, yet not actually be established as causative (Pagano et al. 2004).

Burkitt Lymphoma

Burkitt lymphoma is named after Dr. Dennis Burkitt, an Irish surgeon who while working in Uganda in the 1950s singled out a hitherto unknown tumor of the jaw in children which was remarkable clinically because of the essentially curative responses to treatment with methotrexate. Although untutored in epidemiology he began to suspect a vector-borne infectious cause because of its patterns of occurrence and endemicity. The report of his observations ignited a search for a virus in the tumor. Efforts by the Epstein laboratory in England to detect a virus directly in tumor tissues by electron microscopy were vexing and not fruitful until Dr. Barr, a cytogeneticist, established cultures of cells derived from BL tissue and only then, 50 years ago in 1964, was able to visualize for the first time herpes-like virus particles. That this was a new human herpesvirus was established by serologic studies by the Henle laboratory in Philadelphia.

DNA-DNA nucleic acid hybridization studies by Harald zur Hausen, and soon thereafter by Nonoyama & Pagano, who used their newly devised quantitative cRNA-DNA hybridization assay (1971) for studies of Burkitt lymphoma tissues obtained by George Klein at the Karolinska Institute from Kenya. The analyses soon revealed that more than 98 % were positive for EBV DNA, usually in lower copy numbers consistent with latently infected tumors. Unexpectedly the genome was retained in novel form in the chromatin, the EBV episome, the first found in eukaryotes save for certain plants (1972). Further the viral episomes were retained in constant copy numbers in successive generations of BL cells in culture. Thus the link of the virus to the lymphoma was firmly established genetically (Pagano 2009).

However the occasional specimen was unexpectedly negative for EBV DNA, and testing later of sporadic BL in the United States disclosed that only 14–20 % were positive. These discordant observations were eventually resolved by findings that disruptions & overexpression of the cMyc oncogene produced by the characteristic chromosomal translocations of BL were the common element in both positive & negative BL. This then was the essential molecular lesion that caused BL, not EBV, which acts as a contributory cofactor through its ability to propel growth of B- lymphocytes (Pagano et al. 2004).

In the meantime the Henle's soon confronted a paradox. EBV antibodies were common in healthy children and adults in the United States, but Burkitt lymphoma was then virtually unknown. Moreover they were able to establish cultures of lymphocytes from the peripheral blood of infected, but not from noninfected, persons. The mystery of the origin of the EBV antibodies was solved thanks to the observant Henle technician, who had been unable to culture cells from her own blood, but tried again—successfully this time–after contracting infectious mononucleosis, and she now had EBV antibodies. Proof that EBV causes infectious mononucleosis came from the landmark prospective sero-epidemiologic study of IM by Niederman and colleagues in Yale college students. During the 4 years they were studied, only EBV-negative, but not students who were already seropositive, contracted infectious mononucleosis; there were no instances of discordance. The study was conclusive.

That EBV, a γ-herpesvirus, causes the benign disease infectious mononucleosis is indisputable, based on the conclusive epidemiologic studies backed by molecular evidence. Infection of B lymphocytes by the virus causes proliferation of these cells, which in immunologically normal hosts is checked by robust reactive T-cell responses manifested in peripheral blood as atypical lymphocytosis and are not tumorigenic, whereas EBV-infected B-cells are potentially tumorigenic in immune-incompetent persons. In normal hosts these responses check the expansion of the infected B-cells.

Initially the virus replicates in epithelial cells in the oropharynx, where it is secreted into the saliva & can be transmitted by oral contact. Replication in these infected cells is cytolytic in contrast to the proliferation the virus produces in B-lymphocytes, which it infects almost simultaneously. After the initial wave of proliferation subsides, the viral genome persists as episomes lifelong in a small fraction of infected germinal-center memory B-cells, which may divide & are perpetuated by ambient proliferative stimuli (White et al. 2014).

EBV also infects and is shed from the human cervix, probably by oral sexual contact, where it does not produce known disease, although there is evidence that infectious mononucleosis can be contracted occasionally. Multiple strains of EBV have been identified in the oropharynx, but none specifically in the vaginal track, probably because not investigated.

Other EBV Lymphomas

Lethal EBV-infected B-cell lymphomas can arise in immunocompromised hosts such as recipients of organ transplants, in patients with AIDS, or in children with rare innate genetic disorders such as Duncan's Syndrome. These lymphomas, which begin as reversible B-cell lymphoproliferation, evolve into polyclonal, then lethal monoclonal lymphomas that are directly caused by EBV.

In contrast although EBV is detected in approximately 40 % of Hodgkin's lymphoma (HL) its pathogenic role is ill-defined, but presumably nontrivial: the virus infects the pathognomonic cell type, the Reed-Sternberg cell--which is of B-cell origin. EBV infects R-S cells & expresses a viral protein, LMP2a, on the outer membrane of the R-S cell; thus it is expressed in the pathologic cell type of HL & it is likely to exert oncogenic function (White et al. 2014).

Another perspective comes from epidemiologic studies that linked EBV and generation of some cases of HL. The studies showed that the incidence of HL was somewhat but significantly greater in persons who had had infectious mononucleosis (but not subclinical EBV infection) earlier in life. Since the syndrome of IM results from a transient disruption of the immune system, and salivary shedding of virus can continue for years, perhaps some type of residual immunodeficiency plays a role in the genesis of HL, although this is speculative.

EBV also rarely infects and NK and T lymphocytes and produces natural killer/T cell lymphomas especially in Korea and Japan.

EBV Infection in Epithelial Malignancies

The first studies of epithelial infection were based on the hypothesis that as with the other herpesviruses EBV was likely to have a primary cell type in which it replicated initially & a different secondary cell type in which the virus persisted lifelong in a latent form. Since EBV replicated in the oropharynx the search centered on epithelial cells shed in the oropharynx. *In situ* nucleic acid cytohybridization assays had been devised independently by the zur Hausen & Pagano laboratories for detection & localization of EBV DNA in tissues and cells. E-S Huang, Pagano and colleagues with the use of cRNA-DNA hybridization then detected EBV DNA directly in oropharyngeal cells obtained from students with IM. They found also that EBV could infect epithelial cells growing in organ cultures although inefficiently. These findings identified not only a primary entry point & possible source for the virus, but also a basis for understanding the pathogenesis of nasopharyngeal carcinoma, an epithelial malignancy.

Finally aside from IM itself Hairy Leukoplakia (HLP) of the lateral tongue is the only instance of lytic EBVinfection in the oropharynx. Striking features of the lesions, which are in the squamous epithelium, are the masses of virions in them. HL occurs mostly in patients with AIDS, responds to treatment with Acyclovir, but may recur. HLP is not thought to be a premalignant lesion, in contrast to lesions of the tongue produced by smoking cigars or pipes.

Nasopharyngeal Carcinoma (NPC)

Carcinoma of the posterior nasopharynx is the prime example of an epithelial malignancy in which EBV plays a causative role. It has many distinctive features. The neoplasm arises in the fossa of Rosenmuller in Waldenstrom's ring, but the malignancy has a proclivity for early spread, & it is most often diagnosed after it has invaded cervical lymph nodes. It is the most common EBV malignancy: it is endemic in Southern China & has high incidence in first generation émigrés to other Asian countries & to the West Coast of the U.S., mainly in men of middle age. NPC occurs sporadically in Western countries with an incidence ~1/100 that of endemic regions. Of the three WHO histopathologic types undifferentiated Type III NPC is the most common (~90 % of cases). All 3 types, whether of sporadic or endemic origins, are latently infected with EBV episomes. It is sometimes argued that WHO type 1 NPC, which are keratinizing carcinomas & are rare, are not infected with EBV. However this Type tends to have low genome copy numbers that may be missed. Moreover the first EBV genome cloned from NPC was from Type I tissue (Nancy Raab-Traub) (Pagano 2009; White et al. 2014; Raab-Traub 2005).

Other fascinating epidemiologic features of NPC are its intermediate high incidence in North Africa & its puzzling bimodal age distribution in the teens & young adults as well as in the middle-aged in the endemic regions. Geographically it is the only region where those affected are Caucasian.

These complexities of incidence of NPC have provoked numerous investigations of possible cofactors that contribute to genesis of the malignancy including dietary, environmental, including exposure to & metabolism of nitrosamines, and genetic. And despite the innumerable strains of EBV that have been detected in saliva, no "oncogenic strain" has been identified for NPC or the other EBV malignancies.

Additionally reasons for the highly invasive phenotype characteristic of NPC has been illuminated by studies showing that the principal EBV oncogene, LMP1, induces a host of cellular factors including MMP9, MUC1, FGF2, VEGF, HIF1alpha, Twist & Snail capable of propelling every facet of the complex processes of invasion, metastasis, angiogenesis and transcription. Further, such factors may be transported in exosomes to the tumor microenvironment & promote tumor progression. Thus EBV likely functions not only as etiologic agent, but also in late stages of oncogenesis in the array of tumors in which LMP1 is expressed (Yoshizaki et al. 2005).

Finally there are the striking elevations in IgA antibodies to EBV antigens that arise before & around the time of detection of NPC. Since the antibodies are to viral lytic proteins they suggest that a period of viral reactivation & active viral replication precedes NPC & propels its onset, perhaps rapidly, presumably by virus entering pre-malignant cells with acquired mutations. In any case, even though their origin is obscure the wave of antibodies is useful diagnostically & may herald onset of tumor. Indeed in endemic regions of China otoscopy of asymptomatic men & blind biopsy of the Rosenmuller Fossa are used for screening (Pagano 2009).

Parotid Tumors

Parotid, but not other, salivary gland tumors have high incidence in North American Inuits and are EBV-infected, & features of their histopathology resemble those of NPC. Interestingly parotid tumors in NPC-endemic regions of China also contain monoclonal EBV episomes.

Kaposi's Sarcoma Herpesvirus

Kaposi's sarcoma (KS) had been described as a distinctive disease of the skin in 1872, but its viral etiology was identified only in 1994, the second of the two human gamma herpes viruses. It is primarily a vascular endothelial lesion found in its classic form in European men. It is more common in regions of Africa where it is endemic and a considerably more aggressive malignancy. However Kaposi's sarcoma (KS) vaulted to prominence in dermatologic patients with AIDS (Friedman-Kien) before that syndrome had been defined, became its herald lesion and, later, its HIV etiology ascertained (Gallo, Montagnier) (Sir and Ou 2010). KSHV genomes are detected in almost all cases of KS regardless of geographic origin or severity of disease. Somewhat unexpectedly the virus is the causative agent of primary effusion

lymphoma (PEL), a fatal B cell lymphoma. Cell lines established from PEL are invariably infected with KSHV, but often also co-infected with EBV, which seems to enhance its oncogenic behavior (Bushman et al., 2012). Finally KSHV also causes Multicentric Castleman's Disease, an indolent B-cell lymphoproliferative condition (Raab-Traub 2005; Hayward et al. 2010; Wen and Damania 2009).

The KSHV genome is distinctive because of the homologs to cellular genes it encodes, in contrast to EBV, such as a viral cytokine and an interferon regulatory factor. The latter factor however lacks a DNA-binding domain and thus must partner as a heterodimer with a cellular IRF to exert function. EBV can induce cytokines that enhance oncogenic processes, but not does not encode such proteins. The KSHV K1 gene can immortalize primary endothelial cells and produce angioproliferative KS-like lesions in transgenic mice. Finally both KS and PEL are invasive malignancies, and the KSHV gene product K1 is able to upregulate expression of a matrix metalloproteinase and vascular growth factor (Wen and Damania 2009).

Human Papilloma Viruses

A welter of HPV genotypes began to be recognized in the context of cervical cancer and a loose association began to emerge. It was only when Harold zur Hausen began to discern stereotypic associations of certain genotypes (HPV 16 & 18) with cervical cancer, suggestive of a possible etiologic association, that the subsequent revelatory epidemiologic studies could be designed which would lead to proof that certain types of HPV were causative agents. Since HPV causes warts the laboratory also searched for evidence that HPV might cause skin cancers. Interestingly this suspicion was never verified. However HPV is a cause of anal and vaginal cancer as well as benign genital warts.

HPV has also been implicated as causative agent of a subset of oropharyngeal carcinomas. These cancers of the tongue & tonsil occur in younger patients and have a better prognosis than the cancers associated with smoking cigarettes. Although further definition and proof of etiology awaits conclusive epidemiologic studies HPV is likely causative.

Notably HPVinfection led to creation of type-specific vaccines that could protect against infection with the virus & thus prevent cervical carcinoma. However such prophylactic vaccines have effect on cervical cancer itself or early precancerous changes. Therapeutic vaccines directed against early-stage changes in the cervix are in preclinical trial and have shown considerable promise.

Hepatitis B Virus

Hepatocellular carcinoma is endemic in Asian countries and elsewhere. Taiwanese & American investigators who were at first studying human cytomegalovirus infection, which is highly prevalent in woman in that country and can cause congenital

defects, turned their attention to hepatocellular carcinomas, which they suspected might be caused by a virus. Studies of Woodchuck Hepatitis Virus in the United States by Jesse Summers greatly strengthened suspicions. The virus could cause persistent infection of the liver, leading to hepatic fibrosis and ultimately hepatocellular carcinoma in the animals that paralleled the course of hepatic infection in humans. Fortunately even before the virus itself was identified the HBV S-antigen was invariably detectable in the blood of patients with hepatitis, fibrosis and hepatocellular carcinoma itself. Subsequently Palmer Beasley, working with his colleagues in Taiwan designed a sero-epidemiologic study, results of which essentially proved that HBV caused hepatocellular carcinoma. This landmark study was first to prove that a virus could cause a malignancy.

This remarkable journey of discovery led not only to finding the viral culprit, but later to proof produced by a classic sero-epidemiologic study, also designed by Beasley, that hepatitis B virus vaccine could prevent infection with the virus. This indeed was the first vaccine for a human tumor virus, HPV vaccine being the second and only other example.

HBV itself although it is an RNA virus goes through a double-stranded DNA phase and is able to insert into cellular DNA. Although the integration site is not unique it is believed to cause mutational outcomes leading to hepatocellular carcinoma.

The prevalence of the virus is much higher in Asia and other countries than in the United States or other Western countries, because the high prevalence of infection increases the risk to newborns during parturition & leads to persistent productive infection that is more likely to ensue in hepatitis and ultimately hepatocellular carcinoma.

Hepatitis C Virus

Much more prevalent in the United States than HBV, HCV became widespread through blood transfusions before the virus was discovered. It is an RNA virus that produces persistent productive infection in the liver and frequently leads to the sequence of hepatitis, cirrhosis and an increasing incidence of hepatocellular carcinoma. The key to its oncogenic effects is the persistent lytic infection that generates distinctive tissue responses in the liver. The mechanism of oncogenesis is novel in that the basis is the chronic inflammatory response, which has been much studied, that the virus evokes. HCV's RNA genome does not integrate into the cellular genome. Unlike most tumor viruses hepatitis C virus may no longer be detected when the carcinoma is diagnosed, which emphasizes the importance of the distinctive inflammatory responses. The situation while possibly reminiscent of the so-called "hit and run theory" of etiology, is however not the case with HCV--or any other cancer–because the virus is consistently replicating throughout the pro-oncogenic stages if not always at the very end stage of HCC. There are no known instances of this theory (Sir and Ou 2010).

Finally HCV provides the only oncogenic infection that can be eliminated by antiviral therapy. In very recent studies the single antiviral drug (Gilead) completely eliminates infection of the liver in humans thus blocking generation of fibrosis and carcinoma in this ultimately lethal cancer. Another therapy is now available and appears to be effective, but is composed of four different agents including interferon and (blank) with their known side effects.

Human T-Cell Leukemia Virus

Discovered independently by Robert Gallo in the United States and by Hinuma in Japan this is the first known human retrovirus. The Japanese investigators were studying T-cell leukemias that occurred in families in southern Japan. The virus is found in various parts of the world but its familial incidence in Japan indicates that ATL has a genetic basis. The virus does not spread readily. It produces not only subacute T-cell leukemia in adults, but also lymphomas, some of which infiltrate skin (Pagano 2009). This is the only leukemia linked to & caused by a virus. Neither acute myelogenous leukemias of childhood nor chronic B-lymphocytic leukemias in adults have yielded in a search for possible viral etiology.

Conclusion

No single virus is inherently a tumor virus that causes a malignancy, nor does infection of the stomach with Helicobacter pylori necessarily lead to gastric ulcers or cancer. In most instances the infections caused by the viruses may cause illness but are relatively innocuous. Only some of the factors that lead to an oncogenic course of infection are known. However the range of lethal malignancies to which viruses contribute or cause is broad and impressive, and it comprises the largest group of cancers for which causes or substantial contributory factors are known. Both RNA and DNA viruses are represented, and each virus invokes distinct mechanisms of action with some commonalities. Understanding viral mechanisms is in itself a rewarding quest that can also provide insights into central aspects of cell and molecular biology. There is promise that the pace and excitement of discovery is accelerating, attested by the newly isolated polyomaviruses, most not yet linked to disease. Pragmatically the field has on offer many more challenges than accomplishments, starkly witnessed by their paucity so far: two prophylactic vaccines, no therapeutic vaccine, a single antiviral drug. The time is auspicious; we are still on frontiers at every level when the yield on investment both scientifically and materially can grow.

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