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Introduction

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Human oncogenic viruses: nature and discovery

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Seven kinds of virus collectively comprise an important cause of cancer, particularly in less developed countries and for people with damaged immune systems. Discovered over the past 54 years, most of these viruses are common infections of humankind for which malignancy is a rare consequence. Various cofactors affect the complex interaction between virus and host and the likelihood of cancer emerging. Although individual human tumour viruses exert their malignant effects in different ways, there are common features that illuminate mechanisms of oncogenesis more generally, whether or not there is a viral aetiology.

This article is part of the themed issue 'Human oncogenic viruses'.

1. Introduction

Cancers are not contagious in the sense that they are not transmitted from patients to close contacts. But global studies reveal that about one in six cancers worldwide have an infectious aetiology [1,2]. Although this estimated attributable fraction is significant, it is probably a substantial underestimate since developing countries are particularly hard hit by viral cancers yet tend to have poor or nonexistent cancer registries. Some sites in Africa with high quality reporting, such as Kampala in Uganda [3], reveal that up to 50% of incident cancers are caused by infectious agents and that these cancers afflict a younger population than traditionally seen in North American or European settings. Some cancer registries exclude non-melanoma skin cancers, or attribute deaths to HIV rather than HIV-related cancers [4]. These estimates also tend to exclude new infectious causes for cancer (e.g. Merkel cell polyomavirus) or a new association of a known virus with a tumour; for instance, Epstein–Barr virus (EBV) causes more cases of gastric carcinoma than lymphoma. Various infectious agents, such as bacteria (*Helicobacter pylori* in stomach cancer and mucosal-associated lymphomas) and helminths (carcinomas of the urinary bladder and gall bladder) are associated with malignancy, but approximately 1.6 million of the 2 million new cancer cases each year due to infection arise as a consequence of persistent infection by oncogenic viruses. This theme issue focuses on new developments for those viruses and their mechanisms of action.

The notion that a transmissible agent might play a role in some types of human cancer dates back to epidemiological observations made by the physician Domenico Rigoni-Stern in 1842 [5]. Analysing death certificates for women of Verona in 1760–1839, he noted that while nuns had an increased risk of breast cancer, they had a lower risk of cervical cancer compared to married women and much lower risk than sex workers. He concluded that the development of cervical cancer is related to sexual contact. Of course, there was no knowledge of hormones or viruses in Rigoni-Stern's time, but it seems a neat symmetry that 120 years later, the 1966 Nobel Prize for Physiology and Medicine was awarded on the one hand to Charles Huggins 'For his discoveries concerning hormonal treatment of cancer' and on the other to Peyton Rous 'For his discovery of tumour-inducing viruses'. Isolation of Rous's eponymous sarcoma virus in chickens had been made 55 years earlier, representing the longest 'incubation period' between discovery and recognition

in the annals of the Nobel Prizes [6]. An additional 42 years passed before Harald zur Hausen was similarly recognized for the discovery of strains of human papillomavirus (HPV) that cause cervical cancer [7]. Tumour viruses led the way in early molecular cell biology with Nobel awards to Renato Dulbecco, Howard M Temin and David Baltimore in 1975 for *in vitro* cell transformation by viruses and for reverse transcriptase; to Baruch S Blumberg in 1976 for the elucidation of hepatitis B virus; to J Michael Bishop and Harold E Varmus in 1989 for the cellular origin of retroviral oncogenes; and to Richard Roberts and Philip Sharp in 1993 for the discovery of RNA splicing in adenovirus.

Tumour-inducing viruses have been of immense importance to our understanding of molecular carcinogenesis leading to the discovery of oncogenes and tumour suppressor genes. Oncogenes were first discovered in association with retroviruses and later applied to most forms of cancer [8–10]. Studies on human and animal cancer viruses led to discoveries that include over 30 cellular oncogenes, genes that are prefixed with a 'c' (e.g. c-*myc*, c-*erb*, c-*fos*), whereas oncogenes carried by viruses are prefixed with a 'v' (e.g. v-*myc* and v-*ras*). H-*ras* and K-*ras* were first found as viral oncogenes stolen from the host genome by the Harvey rat sarcoma and Kirsten rat sarcoma viruses respectively whereas N-*ras* was discovered directly in human tumours by DNA transfection. Before mutation analysis became standard, tumour suppressors and oncoproteins such p53 [11,12] and PI3 K [13] were identified by their association with tumour virus proteins. It is not an exaggeration to say that tumour virology formed the bedrock for all areas of modern cancer molecular biology.

Just over half a century has passed since first discovery of a human virus causing cancer, Epstein–Barr virus (EBV or HHV4) [14]. Now there are seven established human cancer viruses (table 1), with additional suspects being scrutinized. In this theme issue, the authors survey cancer viruses and give a broad understanding of the common features and the differences between the viruses. Lunn *et al.* [15] provide an overview on the epidemiology of viral tumours, outlining their common characteristics as well as their differences. Technologies have made enormous progress since 1964 and the complex interplay between viruses and their hosts is being uncovered at the molecular level: viral genomics by Tang & Larsson [16]; innate immune evasion by Hopcraft & Damania [17]; and viral regulation of DNA replication and repair by Pancholi *et al.* [18]. The recognition that viruses can cause cancer has led to two anti-cancer vaccines targeting infection by high-risk strains of human papillomaviruses (HPV) and by hepatitis B virus (HBV), as described by Stanley [19], which have already begun to reduce the incidence of these cancers in humans [20,21].

We have also been fortunate to recruit leading experts for each of the major cancer viruses as authors for reviews. The role of EBV in B-cell tumours is discussed by Shannon-Lowe *et al.* [22] and in undifferentiated nasopharyngeal carcinoma by Tsao *et al.* [23]. The related gamma-herpesvirus, Kaposi sarcoma herpesvirus (KSHV or HHV8), rose to prominence by causing an epidemic of cancer among AIDS patients and remains a scourge in Africa, parts of Asia and parts of South America as described by Mariggiò *et al.* [24]. We turn to the small human DNA viruses with a review by McBride [25] on HPV, emphasizing the novelties of their replication. DeCaprio [26] describes current understanding on the most recently recognized human tumour virus, Merkel cell polyomavirus (MCV), and other newly discovered human polyomaviruses that have been discovered by sequencing technologies. Liver cancer resulting from HBV and hepatitis C viruses (HCV) infection is an unsolved public health problem despite the success of the HBV vaccine and anti-viral drugs targeting HCV. Ringelhan *et al.* [27] describe these liver pathogens and point towards how the fundamental molecular virology of these viruses can provide new avenues for prevention and therapy. Finally, the human retroviruses, viruses with both RNA- and DNA-based life cycles, remain a fundamental source of new biology. Bangham & Matsuoka [28] describe human T lymphotropic virus type I (HTLV-I) while Kassiotis & Stoye [29] discuss endogenous human retroviruses. HIV contributes to each of these viral cancers by targeting the immune system and, because of this, it is considered a human carcinogen by the International Agency for Cancer Research. Given the magnitude of the HIV public health problem, however, it is well-addressed in many other reviews and in chapters in this issue on individual tumour viruses.

The recently-deceased pioneer in tumour virus research, George Klein, together with Ingemar Ernberg likened the field to a roller coaster [30], first going up, up and up, to become the centre piece of President Nixon's US National Cancer Institute initiative in the late 1960s, and then racing down with the newly emerging concept of cellular oncogene-tumour suppressor gene networks, most of which were discovered in studies of animal tumour viruses [9]. In the popular mind, viruses travelled from being the cause for all kinds of cancer to being irrelevant to any cancer. Klein's roller coaster has now edged up to a long and level stretch: a small number of viruses cause a large fraction of human cancer cases, particularly in less-developed nations. Studying these viruses provides new insights into all cancers and the cancer biologist who ignores molecular virology does so at his or her own professional risk.

Each human tumour virus discovery led to new general concepts in medicine and the emergence of cancer immunotherapies and vaccines show that these foreign antigen-containing tumours are low-hanging fruit that can be used as models for immunotherapeutics in non-infectious cancers. Recent studies on innate immunity and the human virome also reveal that the old notion that tumour viruses disable cell-cycle checkpoints only to replicate their own genomes is too simplistic. By better understanding how these viruses interact with the host cell, to target both replication machinery and immune pathways [31–35], we come to a more sophisticated view of the interplay between innate immunity and cellular proliferation controls [36,37]. These insights are directly relevant to cancers whether or not they are caused by a foreign virus.

2. Virus–host interaction

The human tumour viruses (table 1) range from a positively-stranded RNA virus (HCV), to a complex and stable retrovirus (HTLV-I, unlike the majority of animal tumour retroviruses that pirated cellular genes), from small (MCV and HPV) to large DNA viruses (KSHV and EBV), and include a DNA virus with a retroviral component to its life cycle (HBV). In short, they represent many forms of viruses and

Table 1. Viruses involved in human cancer. ca., cancer; ds., disease.

virus	virus discovered	method of discovery	virus classification	malignancy	other disease
Epstein–Barr virus (EBV)	1964	cell culture and electronmicroscopy	*Herpesviridae* dsDNA	Burkitt's lymphoma diffuse large B-cell lymphoma Hodgkin lymphoma undifferentiated nasopharyngeal ca. gastric adenocarcinoma leiomyosarcoma post-transplant lymphoproliferative ds.	infectious mononucleosis X-linked lymphoproliferative syndrome/(Duncan syndrome)
hepatitis B virus (HBV)	1965	serologic screening	*Hepadnaviridae* dsDNA-RT	hepatocellular carcinoma	hepatitis cirrhosis
human T-lymphotropic virus-1 (HTLV-1)	1980	cell culture, RT assay and electromicroscopy	*Retroviridae* ssRNA-RT, positive strand	adult T-cell leukaemia (ATL)	HTLV-1 myelopathy/ tropical spastic paraparesis (HAM/TSP)
human genital papillomavirus (HPV)	1983	DNA hybridization	*Papillomaviridae* dsDNA	cervical carcinoma squamous cell head and neck ca. squamous cell anal cancer vulvar cancer	
hepatitis C virus (HCV)	1989	cDNA library screening	*Flaviviridae* ssRNA-RT, positive strand	hepatocellular carcinoma rare lymphomas?	hepatitis cirrhosis
Kaposi sarcoma herpesvirus (KSHV/HHV8)	1994	representational difference analysis (RDA)	*Herpesviridae* dsDNA	Kaposi's sarcoma primary effusion lymphoma multicentric Castleman ds.	
Merkel cell polyomavirus (MCV)	2008	digital transcriptome subtraction (DTS)	*Polyomaviridae* dsDNA	Merkel cell carcinoma	

it initially seems difficult to describe what they have in common. From a broad perspective, however, common features begin to emerge.

(a) Viral cancers are biological accidents

None of the oncogenic viruses induce tumours as a fundamental part of their viral life cycles. All seven of the viruses cause infection and are transmitted without the majority of infected persons developing neoplasia. While an appealing hypothesis is that the growing mass of a tumour would harbour more infectious virions, this does not turn out to be the case since viruses are almost always present in a non-infectious form in tumours. It appears that viral cancers, similar to non-infectious cancers, are biological accidents and in the natural course of disease the resultant neoplasms are just as deadly to the virus as they are to their hosts. This raises an intriguing teleological question: if viral oncogenes did not evolve to cause cancer to benefit the virus, why are they conserved? For many years it was assumed that these genes simply generated an S-phase cell-cycle state to promote viral nucleic acid replication, and inhibited apoptotic routines that would be initiated from unscheduled cell-cycle entry [38]. More recent discoveries in innate immunology reveal that cells respond to infection by initiating cell-cycle arrest and initiation of programmed cell death [39]. Thus, it appears likely that viral oncogenes also serve as immune evasion genes that prevent the host cell from initiating these stereotypic responses to infection.

(b) Absence of virion production from tumour cells

Early research in tumour virology noted that a common feature of viral tumours—distinct from all other viral diseases—is that these viruses are generally 'non-permissive' for replication within tumour cells [40]. Active virus replication, so called lytic replication, triggers cellular host immune responses that lead to death of the infected cell generating the well-described cytopathic effect. In viral tumours, viruses are retained in each tumour cell in a near silent state (latency) either producing viral oncoproteins or initiating insertional mutagenesis that drive tumour cell proliferation—the possible exception being hepatocellular carcinoma induced by HCV. The importance of non-permissivity to tumorigenesis is seen with HPV, HBV and MCV, agents that do not typically form tumours without mutations and integration events that make it impossible for the virus to actively replicate ('pseudolatency'). For EBV and KSHV, the viruses are in a latent state in most of the cancer cells so that no infectious particles are generated from the bulk of tumour cells. Replication occurring in a minority fraction of tumour cells can produce infectious virions but will kill the initiating tumour cells. This same principle is leveraged by viral oncolytic therapies to treat specific tumours with defects in dual immune-tumour suppressor signalling pathways that are not present in healthy surrounding tissues, making some tumours particularly susceptible to viral lytic infection and induction of an effective anticancer immune response [41].

(c) Viral cancers occur as chronic infections

All of the human cancer viruses are capable of prolonged, persistent infections. For some of these viruses, particularly EBV and MCV, infection is generally thought to be lifelong and widespread in all human populations, so much so that these viruses are part of our 'normal' viral flora. For HBV and HCV, it is likely that smouldering infections are responsible for chronic inflammation and cirrhosis that secondarily lead to liver cancer but these infections nonetheless typically last for decades before cancers emerge. Prolonged viral persistence increases the likelihood for required secondary conditions that change a silent viral infection into a symptomatic cancer. Kaposi's sarcoma in AIDS patients reflects loss of immunologic control over a persistent KSHV infection that usually occurred decades previously.

(d) Target cell specificity of oncogenic viruses

Neoplasia induced by tumour viruses reflect viral cell tropism. For some viruses, this is highly restricted such as HBV, which only causes primary hepatocellular carcinoma but not other cancers. Similarly, HTLV-I causes a specific subtype of $CD4^+$ T-cell tumours called adult T-cell leukaemia (or adult T-cell leukaemia/lymphoma owing to lymphomatous lesions in the skin). In contrast, high-risk human papillomaviruses are limited to squamous epithelial cancers but these can occur at different body sites, including cervical, head-and-neck and anal cancers, that are associated with sites of sexual transmission (heterosexual, orosexual and anosexual activity, respectively). In contrast, both KSHV and EBV have a range of tissue reservoirs from which tumours can arise. KSHV is resident in both endothelial and post-germinal B cells and it causes endothelial Kaposi's sarcoma and a B-cell primary effusion lymphoma as well as a B-cell lymphoproliferative syndrome. EBV, the most ubiquitous of all the human cancer viruses, has the least restriction in the types of cancers that is causes. It is not only linked to B-cell lymphomas, but also nasopharyngeal carcinoma, stomach cancer, T-cell lymphomas, and a rare form of leiomyosarcoma (table 1).

There are certain tumours in which a tumour virus is uniformly present and required for tumorigenesis, e.g. cervical cancer and Kaposi's sarcoma. More commonly, however, viruses only cause a portion of a specific tumour histotype. While both HBV and HCV cause hepatocellular carcinoma, this cancer can also be triggered by alcoholic cirrhosis or exposure to dietary mycotoxins, such as aflatoxin mutagen [42], which can act synergistically with HBV in tumour development [43].

Differences in geographical conditions markedly affect the underlying epidemiologies of viral cancers. All cases of Burkitt's lymphoma feature chromosome translocation between c-*myc* on chromosome 8 and one of the immunoglobulin loci on chromosome 14 (IgG heavy chain), chromosome 2 (kappa light chain) or chromosome 22 (lambda light chain), indicative for a required cellular contribution to Burkitt's lymphomagenesis. In North American adults, including Burkitt's lymphoma in AIDS, less than 50% of tumours are EBV positive. In Papua New Guinea and sub-Saharan Africa where malaria is a risk factor for childhood Burkitt's lymphoma, however, EBV is nearly uniformly present in the tumour cells.

For Merkel cell carcinoma, both MCV-positive (approx. 80%) and MCV-negative (approx. 20%) forms exist [44]. MCV-positive tumours tend to be somewhat less aggressive and more responsive to therapy. These tumours also have no consistent pattern of cellular mutations that would suggest a cellular driver mutation, whereas MCV-negative

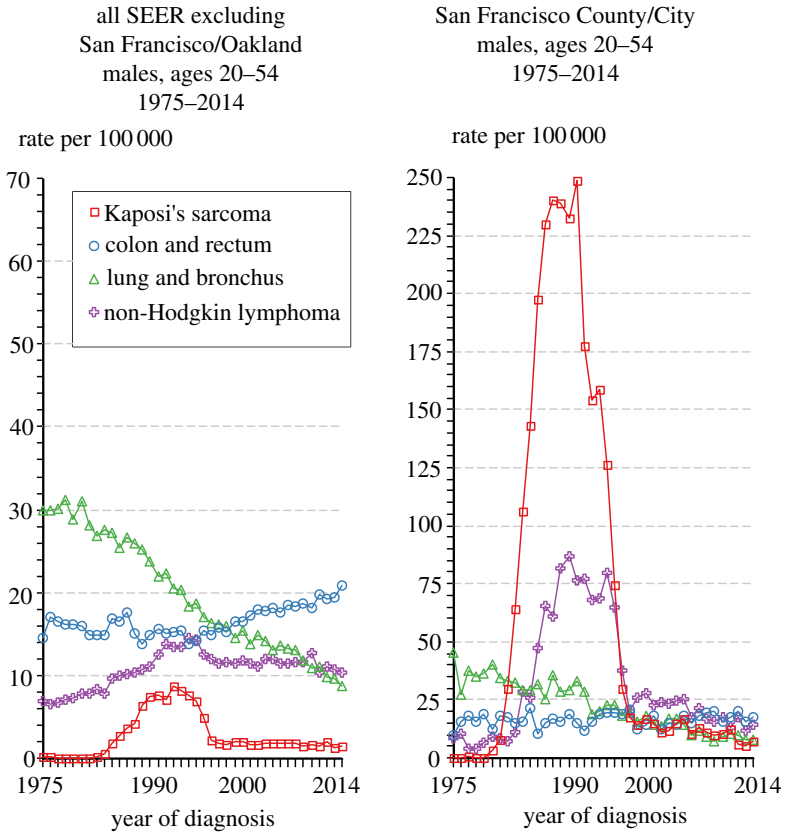

Figure 1. Comparison of an infectious (Kaposi's sarcoma, KS) and a non-infectious cancer (lung and bronchus carcinoma), revealing the impact of the AIDS pandemic. These rates were collected by the US National Cancer Institute's Surveillance, Epidemiology and End-Results (SEER) cancer registry program for San Francisco County and City among males aged 20–54 years old. By 1990, epidemic KS rose approximately 240-fold over pre-AIDS era KS rates and declined in subsequent years, especially with the introduction of highly-active antiretroviral therapies in the mid-1990s. Modified from Howlader *et al*. [48]. (Online version in colour.)

tumours show a pattern of widespread ultraviolet light (UV)-induced genomic mutations [45]. It has been postulated that the carcinogenic load caused by an integrated and mutated MCV genomic is biologically equivalent to 10 000 s of random genomic UV mutations [46].

(e) Immune control of viral tumours

Nearly all viral cancers that have been described so far have increased incidence among immunosuppressed persons [47] (figure 1). This is particularly evident for those viruses that directly transform cells (HTLV-I, HPV, MCV, EBV and KSHV) by expression of foreign oncogenes. Thus, cancer registry studies of AIDS and transplant patients have been critical in identifying potential infectious cancers and contributed directly to discovery of tumour viruses, including KSHV [49] and MCV [50]. Furthermore, ageing is associated with more subtle immune dysfunctions and a number of tumours with viral aetiology are associated with advanced age.

(f) All viral cancers have non-infectious cofactors

Non-epidemiologists are sometimes perplexed by viruses as causes for cancer since the vast majority of these infections do not lead to tumours. Infectious disease specialists have long known that exposures to many infections, with exceptions such as rabies virus, smallpox virus and HIV, are often asymptomatic and therefore undetected. Non-infectious cofactors, including age, genetics, environmental factors and prior immunity, are all largely responsible for determining whether exposure to a virus leads to disease. This same principle holds true for infectious cancers. Immunodeficiency, as already described, is a common determinant for whether or not a tumour virus infection will evolve into a cancer. For some of the tumour viruses, host mutations predispose to tumour formation after infection, such as in genes encoding EVER1 and EVER2 for beta HPV-related epidermodysplasia verruciformis [51] or in *SH2D1A* for EBV-related X-linked lymphoproliferative disorder [52]. On the other hand, environmental exposures, such as dietary aflatoxin for HBV-related hepatocellular carcinoma [43] and malaria in childhood Burkitt's lymphoma [53], elevate risk of tumour penetrance after tumour virus exposure.

(g) Human tumour viruses versus rumour viruses

Discovery of a new suspect cancer virus is only the first step in revealing whether or not it is a cause for cancer. In some cases, such as KSHV, a causal link can be relatively quickly determined [54]. For EBV, however, over three decades passed between its discovery and the accumulation of sufficient evidence to sway the scientific community that EBV is indeed a cause of cancer [55]. Unfortunately, epidemiologic causality is often poorly equipped to establish a causal framework of a tumour virus aetiology, particularly when a tumour virus is a component of our natural viral flora [56].

Hunting for tumour viruses has given rise to many spurious claims, especially in the era of PCR—a technique that is simple to use but notorious for contamination and false positivity. SV40 is perhaps the best example of this and has been pursued as a cause for human cancers since its discovery in the early 1960s [57]. But careful analyses over decades fail

to reveal any convincing evidence for SV40 playing a role in human cancer [58]. Antibody cross-reactivity, in both serologic testing and in tissue antigen detection, to known viruses and to viruses yet-to-be discovered, are also common sources for experimental mis-attribution in tumour virology [59,60].

With the advent of high-throughput sequencing, errors at the levels of the parts per billion can also lead to spurious associations. Presence of low-level HPV18 DNA sequences in The Cancer Genome Anatomy (TCGA) sequence database have been traced back to sequencing contaminating HeLa cells [61]. Sequencing-based discovery of a 'new human hepatitis virus' [62] actually turned out to be caused by retained RNA fragments from a marine phytoplankton virus adhering to diatomaceous earth (made from beach sand) used to make RNA isolation spin columns [63]. Endogenous retroviruses also provide rich opportunities to deceive scientists [64]: different viruses discovered in cell lines and naturally suspected to cause rhabdosarcoma and prostate cancer each represented independent examples in which endogenous retroviruses jumped from the host to a cell line when the cancer cells were passed through experimental animals [65]. Uncertainty still exists as to whether Peyton Rous discovered a pre-existing virus that caused the original chicken tumour or if this agent was accidentally and secondarily amplified by the experimental transplantation techniques [6,39] that Rous used to isolate the transforming virus.

New technologies promise to uncover new tumour virus suspects but will also provide opportunities for mistakes and scientific confusion. Simple scientific rigour and a healthy level of scepticism remain among the most important tools for any tumour virologist.

(h) Viral cancers are predominantly a public health burden in developing countries

While wealthy nations in Europe and North America generally have low viral cancer burdens, infection can cause over one-half of cancers in some developing countries. The types of cancer in developing countries, however, vary widely and are influenced by concurrent patterns of AIDS immunosuppression. Among persons from sub-Saharan Africa, who have historically had high endemic KSHV infection prevalence, the HIV pandemic led to an epidemic of AIDS-KS [66]. Other KSHV-endemic areas include western China [67] and Andean South America. In contrast, KSHV and KS are relatively rare for most Southeastern Asians but these same populations have high rates of EBV-related nasopharyngeal and stomach cancers [23] and HBV-related hepatocellular carcinoma [68].

3. Pathways to discovery and detection of human oncogenic viruses

(a) Epidemiology

The pattern of incidence of certain types of tumour may provide an indication that a tumour has an infection as part of its aetiology. The unusual geographical distribution of Burkitt's lymphoma described by Dennis Burkitt led Sir Anthony Epstein in turn to search for a virus. In fact, the causative virus that he discovered [69] is nearly ubiquitous worldwide and the unique geographical distribution of Burkitt's lymphoma is more closely related to associated environmental factors such as malaria prevalence discussed above. Likewise, place of birth studies for patients with adult T-cell leukaemia in Japan led Takatsuki and colleagues [70] to suggest that there may be a transmissible component which after the discovery of HTLV-1 in USA [71] turned out to be that virus [72].

Given the dependence of viral tumour expression on immune suppression, epidemiologic studies of AIDS and transplant patients have been a rich source for identifying new infectious cancers. Analysis of US AIDS patient data by Beral *et al.* [49] provided a detailed epidemiologic description of the likely 'KS agent' and led directly to the discovery of KSHV using genomic subtractive methods [73]. Similarly, elevated rates of Merkel cell carcinoma among AIDS patients compared to the general population [50] provided the basis for a transcriptomic search that identified Merkel cell polyomavirus [74]. The natural history of chronic viral hepatitis preceding onset of hepatocellular carcinoma was a key feature in the identification of HBV and HCV as causes for liver cancer [75,76].

(b) Detection of virus particles

Epstein, Achong and Barr discovered the virus causing Burkitt's lymphoma by electron microscopy (EM) [69], but for reasons already described, this is the least promising pathway to virus detection. Poiesz *et al.* [71] searched for reverse transcriptase activity in the culture supernatants of a cutaneous T-cell lymphoma, now recognized to be a form of adult T-cell leukaemia, and then confirmed the presence of type C retroviral particles (HTLV-1) by electron microscopy.

(c) Detection of viral antigens and antibodies

Markers for virus infection have sometimes been discovered before the viruses themselves and helped to lead to their discovery. The classic example is Baruch Blumberg's delineation of Australia antigen in the 1960s which he first interpreted as a genetic blood group. Later it was discovered by Harvey Alter and Blumberg, and also by Alfred Prince, to be the surface antigen of HBV [77,78]. Similarly, a link between nasopharyngeal carcinoma and EBV was first found by serologic studies [79]. As Louis Pasteur famously observed, chance favours the prepared mind, and these investigators soon realized the significance of their unexpected results.

(d) Viral nucleic acid detection

The non-permissivity of tumour viruses led Harald zur Hausen to focus on identifying viral nucleic acids in tumour tissues rather than encapsidated virions [80]. Knowing that genital wart HPV type 6 had similar epidemiologic features to cervical cancer, he and his colleagues used 'detuned' Southern hybridization with HPV-6 DNA to isolate cross-reactive HPV-16 DNA from a cervical tumour [7] and then HPV-18 from tumour and HeLa cells [81]. With this breakthrough, it became apparent that discovering tumour viruses was more a matter of gene hunting rather than virus hunting.

As with discovery of high-risk HPV, careful epidemiologic studies would be central to subsequent tumour virus discoveries. By 1989, it was clear that a substantial fraction of chronic hepatitis cases had a nonA and nonB viral origin

that could be transmitted in primate animal models. Houghton and colleagues [82] analysed a chimpanzee that had been infected with human nonA, nonB hepatitis serum and generated a cDNA library from its plasma. Using well-characterized human sera from a nonA, nonB case, they searched for cDNA clones that when expressed were specifically reactive to the sera and they succeeded in isolating RNA fragments of hepatitis C virus, which quickly led to full-length viral genome cloning.

Identification of Kaposi's sarcoma herpesvirus (KSHV) in 1994 also depended on isolating cancer-associated genes belonging to a new virus. Applying subtractive representational difference analysis [83] to Kaposi's sarcoma tumours, Chang and colleagues isolated herpesvirus DNA fragments from the tumour which were not present in healthy tissues from the same patient [73]. This new human herpesvirus (KSHV or HHV8) was rapidly shown to fulfil the epidemiologic predictions made for the 'KS agent' and also to cause primary effusion lymphoma [84] and multicentric Castleman's disease [85].

With completion of the human genome and advent of inexpensive sequencing technologies, zur Hausen's strategy for tumour virus discovery could be directly approached. Based on the assumption that any directly-transforming tumour virus will express foreign oncoprotein-encoding mRNA in each tumour cell, Feng *et al*. [86] developed a method to computationally subtract known human sequences from a tumour transcriptome (called digital transcriptome subtraction), which was used to detect Merkel cell polyomavirus DNA from a Merkel cell tumour [74].

The decade following discovery of MCV witnessed an explosion in cancer genomic sequencing, as described by Tang and Larsson Lekholm in this issue [16], and yet no new cancer virus suspects have been uncovered. Time will tell whether we have exhausted the repertoire of human cancer viruses or if there are additional suspects that have been missed. Perhaps, some cancers harbour an agent that is so distant from known viruses that we cannot distinguish it from junk sequences that are in the discard folders on sequencing computers. But MCV also showed that we live with a complex and largely hidden virome that contributes to our health, by training portions of our immune system [87], or can cause cancer when a floral virus undergoes a precise set of mutations [88]. This issue of the Philosophical Transactions explores recent discoveries in human tumour virology, how these cancers can be prevented and controlled, and suggests that Klein's roller coaster may be headed up again.

Data accessibility. This article has no additional data.

Competing interests. We declare we have no competing interests.

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References

1. Parkin DM. 2006 The global health burden of infection-associated cancers in the year 2002. *Int. J. Cancer* **118**, 3030–3044. (doi:10.1002/ijc.21731)
2. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi S. 2016 Global burden of cancers attributable to infections in 2012: a synthetic analysis. *Lancet Glob. Health* **4**, e609–e616. (doi:10.1016/S2214-109X(16)30143-7)
3. Wabinga HR, Parkin DM, Nambooze S. 2012 *Kampala Cancer Registry Report for the period 2007–2009*. Kampala, Uganda: Kampala Cancer Registry.
4. Dittmer DP, Krown SE, Mitsuyasu R. 2017 Exclusion of Kaposi sarcoma from analysis of cancer burden. *JAMA Oncol.* (doi:10.1001/jamaoncol.2016.7092)
5. Scotto J, Bailar III JC. 1969 Rigoni-Stern and medical statistics. A nineteenth-century approach to cancer research. *J. Hist. Med. Allied Sci.* **24**, 65–75. (doi:10.1093/jhmas/XXIV.1.65)
6. Weiss RA, Vogt PK. 2011 100 years of Rous sarcoma virus. *J. Exp. Med.* **208**, 2351–2355. (doi:10.1084/jem.20112160)
7. Durst M, Gissmann L, Ikenberg H, zur Hausen H. 1983 A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc. Natl Acad. Sci. USA* **80**, 3812–3815. (doi:10.1073/pnas.80.12.3812)
8. Coffin JM *et al*. 1981 Proposal for naming host cell-derived inserts in retrovirus genomes. *J. Virol.* **40**, 953–957.
9. Vogt PK. 2012 Retroviral oncogenes: a historical primer. *Nat. Rev. Cancer* **12**, 639–648. (doi:10.1038/nrc3320)
10. Stehelin D, Varmus HE, Bishop JM, Vogt PK. 1976 DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* **260**, 170–173. (doi:10.1038/260170a0)
11. Lane DP, Crawford LV. 1979 T antigen is bound to a host protein in SV40-transformed cells. *Nature* **278**, 261–263. (doi:10.1038/278261a0)
12. Linzer DI, Levine AJ. 1979 Characterization of a 54 K Dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* **17**, 43–52. (doi:10.1016/0092-8674(79)90293-9)
13. Whitman M, Kaplan DR, Schaffhausen B, Cantley L, Roberts TM. 1985 Association of phosphatidylinositol kinase activity with polyoma middle-T competent for transformation. *Nature* **315**, 239–242. (doi:10.1038/315239a0)
14. Lieberman PM. 2014 Epstein-Barr virus turns 50. *Science* **343**, 1323–1325. (doi:10.1126/science.1252786)
15. Lunn RM, Jahnke GD, Rabkin CS. 2017 Tumour virus epidemiology. *Phil. Trans. R. Soc. B* **372**, 20160266. (doi:10.1098/rstb.2016.0266)
16. Tang K-W, Larsson E. 2017 Tumour virology in the era of high-throughput genomics. *Phil. Trans. R. Soc. B* **372**, 20160265. (doi:10.1098/rstb.2016.0265)
17. Hopcraft SE, Damania B. 2017 Tumour viruses and innate immunity. *Phil. Trans. R. Soc. B* **372**, 20160267. (doi:10.1098/rstb.2016.0267)
18. Pancholi NJ, Price AM, Weitzman MD. 2017 Take your PIKK: tumour viruses and DNA damage response pathways. *Phil. Trans. R. Soc. B* **372**, 20160269. (doi:10.1098/rstb.2016.0269)
19. Stanley M. 2017 Tumour virus vaccines: hepatitis B virus and human papillomavirus. *Phil. Trans. R. Soc. B* **372**, 20160268. (doi:10.1098/rstb.2016.0268)
20. Chang MH, Chen CJ, Lai MS, Hsu HM, Wu TC, Kong MS, DC Liang DC, Shau WY, Chen DS. 1997 Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N. Engl. J. Med.* **336**, 1855–1859. (doi:10.1056/NEJM199706263362602)
21. Schiller JT, Castellsague X, Garland SM. 2012 A review of clinical trials of human papillomavirus prophylactic vaccines. *Vaccine* **30**(Suppl. 5), F123–F138. (doi:10.1016/j.vaccine.2012.04.108)
22. Shannon-Lowe C, Rickinson AB, Bell AI. 2017 Epstein–Barr virus-associated lymphomas. *Phil. Trans. R. Soc. B* **372**, 20160271. (doi:10.1098/rstb.2016.0271)

23. Tsao SW, Tsang CM, Lo KW. 2017 Epstein–Barr virus infection and nasopharyngeal carcinoma. *Phil. Trans. R. Soc. B* **372**, 20160270. (doi:10.1098/rstb.2016.0270)
24. Mariggiò G, Koch S, Schulz TF. 2017 Kaposi sarcoma herpesvirus pathogenesis. *Phil. Trans. R. Soc. B* **372**, 20160275. (doi:10.1098/rstb.2016.0275)
25. McBride AA. 2017 Oncogenic human papillomaviruses. *Phil. Trans. R. Soc. B* **372**, 20160273. (doi:10.1098/rstb.2016.0273)
26. DeCaprio JA. 2017 Merkel cell polyomavirus and Merkel cell carcinoma. *Phil. Trans. R. Soc. B* **372**, 20160276. (doi:10.1098/rstb.2016.0276)
27. Ringehan M, McKeating JA, Protzer U. 2017 Viral hepatitis and liver cancer. *Phil. Trans. R. Soc. B* **372**, 20160274. (doi:10.1098/rstb.2016.0274)
28. Bangham CRM, Matsuoka M. 2017 Human T-cell leukaemia virus type 1: parasitism and pathogenesis. *Phil. Trans. R. Soc. B* **372**, 20160272. (doi:10.1098/rstb.2016.0272)
29. Kassiotis G, Stoye JP. 2017 Making a virtue of necessity: the pleiotropic role of human endogenous retroviruses in cancer. *Phil. Trans. R. Soc. B* **372**, 20160277. (doi:10.1098/rstb.2016.0277)
30. Ernberg I, Klein G. 2007 Effects on apoptosis, cell cycle and transformation, and comparative aspects of EBV with other DNA tumor viruses. In *Human herpesviruses: biology, therapy and immunoprophylaxis* (eds A Arvin, G Campadelli-Fiume, E Mocarski, PS Moore, B Roizman, R Whitley, K Yamanishi), pp. 514–539. Cambridge, UK: Cambridge University Press.
31. Stracker TH, Carson CT, Weitzman MD. 2002 Adenovirus oncoproteins inactivate the Mre11-Rad50-NBS1 DNA repair complex. *Nature* **418**, 348–352. (doi:10.1038/nature00863)
32. Zhang G, Chan B, Samarinan N, Abere B, Weidner-Glunde M, Buch A, Pich A, Brinkmann MM, Schulz TF. 2016 Cytoplasmic isoforms of Kaposi sarcoma herpesvirus LANA recruit and antagonize the innate immune DNA sensor cGAS. *Proc. Natl Acad. Sci. USA* **113**, E1034–E1043. (doi:10.1073/pnas.1516812113)
33. Lau L, Gray EE, Brunette RL, Stetson DB. 2015 DNA tumor virus oncogenes antagonize the cGAS-STING DNA-sensing pathway. *Science* **350**, 568–571. (doi:10.1126/science.aab3291)
34. Ma Z *et al.* 2015 Modulation of the cGAS-STING DNA sensing pathway by gammaherpesviruses. *Proc. Natl Acad. Sci. USA* **112**, E4306–E4315. (doi:10.1073/pnas.1503831112)
35. Stavrou S, Blouch K, Kotla S, Bass A, Ross SR. 2015 Nucleic acid recognition orchestrates the anti-viral response to retroviruses. *Cell Host Microbe* **17**, 478–488. (doi:10.1016/j.chom.2015.02.021)
36. Moore PS, Chang Y. 1998 Antiviral activity of tumor-suppressor pathways: clues from molecular piracy by KSHV. *Trends Genet.* **14**, 144–150. (doi:10.1016/S0168-9525(98)01408-5)
37. Moore PS, Chang Y. 2003 Kaposi's sarcoma-associated herpesvirus immunoevasion and tumorigenesis: two sides of the same coin? *Annu. Rev. Microbiol.* **57**, 609–639. (doi:10.1146/annurev.micro.57.030502.090824)
38. Levine AJ. 2009 The common mechanisms of transformation by the small DNA tumor viruses: the inactivation of tumor suppressor gene products: p53. *Virology* **384**, 285–293. (doi:10.1016/j.virol.2008.09.034)
39. Moore PS, Chang Y. 2010 Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat. Rev. Cancer* **10**, 878–889. (doi:10.1038/nrc2961)
40. Zur Hausen H. 2009 The search for infectious causes of human cancers: where and why. *Virology* **392**, 1–10. (doi:10.1016/j.virol.2009.06.001)
41. Guo ZS, Liu Z, Kowalsky S, Feist M, Kalinski P, Lu B, Storkus WJ, Bartlett DL. 2017 Oncolytic immunotherapy: conceptual evolution, current strategies, and future perspectives. *Front. Immunol.* **8**, 555. (doi:10.3389/fimmu.2017.00555)
42. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. 2006 The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J. Hepatol.* **45**, 529–538. (doi:10.1016/j.jhep.2006.05.013)
43. Kirk GD, Bah E, Montesano R. 2006 Molecular epidemiology of human liver cancer: insights into etiology, pathogenesis and prevention from the Gambia, West Africa. *Carcinogenesis* **27**, 2070–2082. (doi:10.1093/carcin/bgl060)
44. Chang Y, Moore PS. 2012 Merkel cell carcinoma: a virus-induced human cancer. *Annu. Rev. Pathol.* **7**, 123–144. (doi:10.1146/annurev-pathol-011110-130227)
45. Sihto H, Kukko H, Koljonen V, Sankila R, Bohling T, Joensuu H. 2009 Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. *J. Natl Cancer Inst.* **101**, 938–945. (doi:10.1093/jnci/djp139)
46. Starrett GJ *et al.* 2017 Merkel cell polyomavirus exhibits dominant control of the tumor genome and transcriptome in virus-associated Merkel cell carcinoma. *MBio* **8**, e02079-16. (doi:10.1128/mBio.02079-16)
47. Grulich AE, Vajdic CM. 2015 The epidemiology of cancers in human immunodeficiency virus infection and after organ transplantation. *Semin. Oncol.* **42**, 247–257. (doi:10.1053/j.seminoncol.2014.12.029)
48. Howlader N *et al.* (eds). 2017 *SEER Cancer Statistics Review, 1975–2014*. Bethesda, MD: National Cancer Institute. https://seer.cancer.gov/csr/1975_2014/, based on November 2016 SEER data submission, posted to the SEER web site, April 2017.
49. Beral V, Peterman TA, Berkelman RL, Jaffe HW. 1990 Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection? *Lancet* **335**, 123–128. (doi:10.1016/0140-6736(90)90001-L)
50. Engels EA, Frisch M, Goedert JJ, Biggar RJ, Miller RW. 2002 Merkel cell carcinoma and HIV infection. *Lancet* **359**, 497–498. (doi:10.1016/S0140-6736(02)07668-7)
51. Tommasino M. 2017 The biology of beta human papillomaviruses. *Virus Res.* **231**, 128–138. (doi:10.1016/j.virusres.2016.11.013)
52. Veillette A, Perez-Quintero LA, Latour S. 2013 X-linked lymphoproliferative syndromes and related autosomal recessive disorders. *Curr. Opin Allergy Clin. Immunol.* **13**, 614–622. (doi:10.1097/ACI.0000000000000008)
53. Moormann AM, Bailey JA. 2016 Malaria—how this parasitic infection aids and abets EBV-associated Burkitt lymphomagenesis. *Curr. Opin. Virol.* **20**, 78–84. (doi:10.1016/j.coviro.2016.09.006).
54. Moore PS, Chang Y. 2014 The conundrum of causality in tumor virology: the cases of KSHV and MCV. *Semin. Cancer Biol.* **26**, 4–12. (doi:10.1016/j.semcancer.2013.11.001)
55. International Agency for Cancer Research (IARC). 1997 *Epstein-Barr virus and Kaposi's sarcoma herpesvirus/human herpesvirus 8*, vol. 70. Lyon, France: World Health Organization.
56. Moore PS, Chang Y. 2017 Common commensal cancer viruses. *PLoS Pathog.* **13**, e1006078. (doi:10.1371/journal.ppat.1006078)
57. zur Hausen H. 2003 SV40 in human cancers—an endless tale? *Int. J. Cancer* **107**, 687. (doi:10.1002/ijc.11517)
58. Poulin DL, DeCaprio JA. 2006 Is there a role for SV40 in human cancer? *J. Clin. Oncol.* **24**, 4356–4365. (doi:10.1200/JCO.2005.03.7101)
59. Viscidi RP, Clayman B. 2006 Serological cross reactivity between polyomavirus capsids. *Adv. Exp. Med. Biol.* **577**, 73–84. (doi:10.1007/0-387-32957-9_5)
60. DeCaprio JA, Garcea RL. 2013 A cornucopia of human polyomaviruses. *Nat. Rev. Microbiol.* **11**, 264–276. (doi:10.1038/nrmicro2992)
61. Cantalupo PG, Katz JP, Pipas JM. 2015 HeLa nucleic acid contamination in the cancer genome atlas leads to the misidentification of human papillomavirus 18. *J. Virol.* **89**, 4051–4057. (doi:10.1128/JVI.03365-14)
62. Xu B *et al.* 2013 Hybrid DNA virus in Chinese patients with seronegative hepatitis discovered by deep sequencing. *Proc. Natl Acad. Sci. USA* **110**, 10 264–10 269. (doi:10.1073/pnas.1303744110)
63. Naccache SN, Hackett Jr J, Delwart EL, Chiu CY. 2014 Concerns over the origin of NIH-CQV, a novel virus discovered in Chinese patients with seronegative hepatitis. *Proc. Natl Acad. Sci. USA* **111**, E976. (doi:10.1073/pnas.1317064111)
64. Voisset C, Weiss RA, Griffiths DJ. 2008 Human RNA 'rumor' viruses: the search for novel human retroviruses in chronic disease. *Microbiol. Mol. Biol. Rev.* **72**, 157–196. (doi:10.1128/MMBR.00033-07)
65. Paprotka T *et al.* 2011 Recombinant origin of the retrovirus XMRV. *Science* **333**, 97–101. (doi:10.1126/science.1205292)
66. Cook-Mozaffari P, Newton R, Beral V, Burkitt DP. 1998 The geographical distribution of Kaposi's sarcoma and of lymphomas in Africa before the AIDS epidemic. *Br. J. Cancer* **78**, 1521–1528. (doi:10.1038/bjc.1998.717)
67. Fu B *et al.* 2009 Seroprevalence of Kaposi's sarcoma-associated herpesvirus and risk factors in

Xinjiang, China. *J. Med. Virol.* **81**, 1422–1431. (doi:10.1002/jmv.21550)

68. El-Serag HB, Rudolph KL. 2007 Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* **132**, 2557–2576. (doi:10.1053/j.gastro.2007.04.061)
69. Epstein MA, Achong BG, Barr YM. 1964 Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet* **15**, 702–703. (doi:10.1016/S0140-6736(64)91524-7)
70. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. 1977 Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood* **50**, 481–492.
71. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. 1980 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. USA* **77**, 7415–7419. (doi:10.1073/pnas.77.12.7415)
72. Miyoshi I, Kubonishi I, Yoshimoto S, Akagi T, Ohtsuki Y, Shiraishi Y, Nagata K, Hinuma Y. 1981 Type C virus particles in a cord T-cell line derived by co-cultivating normal human cord leukocytes and human leukaemic T cells. *Nature* **294**, 770–771. (doi:10.1038/294770a0)
73. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. 1994 Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* **265**, 1865–1869. (doi:10.1126/science.7997879)
74. Feng H, Shuda M, Chang Y, Moore PS. 2008 Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* **319**, 1096–1100. (doi:10.1126/science.1152586)
75. Beasley RP, Hwang LY, Lin CC, Chien CS. 1981 Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. *Lancet* **2**, 1129–1133. (doi:10.1016/S0140-6736(81)90585-7)
76. Sbolli G, Zanetti AR, Tanzi E, Cavanna L, Civardi G, Fornari F, Di Stasi M, Buscarini L. 1990 Serum antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *J. Med. Virol.* **30**, 230–232. (doi:10.1002/jmv.1890300316)
77. Blumberg BS, Alter HJ, Visnich S. 1965 A 'new' antigen in leukemia sera. *JAMA* **191**, 541–546. (doi:10.1001/jama.1965.03080070025007)
78. Ganem D, Prince AM. 2004 Hepatitis B virus infection—natural history and clinical consequences. *N. Engl. J. Med.* **350**, 1118–1129. (doi:10.1056/NEJMra031087)
79. Henle W *et al.* 1970 Antibodies to Epstein-Barr virus in nasopharyngeal carcinoma, other head and neck neoplasms, and control groups. *J. Natl Cancer Inst.* **44**, 225–231.
80. zur Hausen H. 2009 Papillomaviruses in the causation of human cancers—a brief historical account. *Virology* **384**, 260–265. (doi:10.1016/j.virol.2008.11.046)
81. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H. 1984 A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. *EMBO J.* **3**, 1151–1157.
82. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. 1989 Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* **244**, 359–362. (doi:10.1126/science.2523562)
83. Lisitsyn N, Lisitsyn N, Wigler M. 1993 Cloning the differences between two complex genomes. *Science* **259**, 946–951. (doi:10.1126/science.8438152)
84. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. 1995 Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *N. Engl. J. Med.* **332**, 1186–1191. (doi:10.1056/NEJM199505043321802)
85. Soulier J *et al.* 1995 Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. *Blood* **86**, 1276–1280.
86. Feng H, Taylor JL, Benos PV, Newton R, Waddell K, Lucas SB, Chang Y, Moore PS. 2007 Human transcriptome subtraction by using short sequence tags to search for tumor viruses in conjunctival carcinoma. *J. Virol.* **81**, 11 332–11 340. (doi:10.1128/JVI.00875-07)
87. Barton ES, White DW, Cathelyn JS, Brett-McClellan KA, Engle M, Diamond MS, Miller VL, Virgin HW. 2007 Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* **447**, 326–329. (doi:10.1038/nature05762)
88. Shuda M, Feng H, Kwun HJ, Rosen ST, Gjoerup O, Moore PS, Chang Y. 2008 T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. *Proc. Natl Acad. Sci. USA* **105**, 16 272–16 277. (doi:10.1073/pnas.0806526105)