ELSEVIER

European Journal of Cancer 40 (2004) 1998–2005

European Journal of Cancer

www.ejconline.com

Viruses and tumours – an update

Simon J. Talbot *, Dorothy H. Crawford

Centre for Infectious Diseases, University of Edinburgh, R(D)SVS, Summerhall, Edinburgh EH9 1QH, UK

Received 10 September 2003; accepted 12 November 2003
Available online 19 June 2004

Abstract

In the 40 years since the publication of the article by Harris (*Eur J Cancer* 1965, **1**, 183–188), considerable progress has been made towards the understanding of the contribution of infectious agents to the development of tumours. It is estimated that 15–20% of human malignancies may have an infectious aetiology. This article attempts to summarise the current level of our knowledge with respect to tumour-associated viruses, and to place this in the context of the earlier article by Harris (*Eur J Cancer* 1965, **1**, 183–188).

Keywords: Viruses; Cancer; Molecular biology; Herpesviruses; Casuality

1. Introduction

Since the publication of the article by Harris [1], many important discoveries relating to cancer epidemiology and to the carcinogenic effects of micro-organisms have been reported (Table 1). Approximately one-fifth of all human cancers worldwide arise in the stomach (9%), liver (6%) or cervix (5%), and most of these would be prevented if these infections could be eradicated [2]. More than 100 human papillomaviruses (HPVs) have been sequenced, and DNA from a phylogenetic subgroup of sexually transmitted HPVs that includes HPV16, HPV18 and HPV45 is detectable in virtually all cervical cancers worldwide [3]. These and other HPVs are also found in other anogenital cancers and may also cause cancers of other sites (head and neck, oesophagus and skin) [4]. The contribution of hepatitis-B virus (HBV) to hepatocellular carcinoma (HCC) in high-incidence regions has long been recognised although the synergistic effect of smoking is a more recent discovery [5]. The hepatitis-C virus (HCV) is similarly carcinogenic [5].

Other viruses that present a substantial cancer risk in certain populations include Epstein–Barr virus (EBV; associated with various lymphoid malignancies and nasopharyngeal cancer), human T-cell lymphotropic virus type-1 (HTLV-1; adult T-cell leukaemia/lymphoma), human immunodeficiency virus (HIV; non-Hodgkin's lymphoma), human herpesvirus-8 (Kaposi's sarcoma) [6,7]. There is also strong epidemiological evidence for an infective aetiology in childhood leukaemia [8], but no specific pathogen has been implicated. The incidence of several virally-induced cancers is further increased by specific cofactors such as dietary aflatoxin (liver), salted fish (nasopharynx) and smoking (liver and cervix).

2. DNA viruses and tumour suppressor proteins

Much knowledge has accumulated over the past 40 years on the molecular biology of cell transformation by oncogenic DNA viruses. Indeed, it was through the study of viruses that important tumour suppressor proteins such as p53 were first identified [9].

The genes responsible for transformation in DNA viruses have no cellular homologues. They are true viral genes required for viral growth. Most cells in the body are quiescent and not synthesising DNA for replication. DNA viruses need to make DNA in order to replicate and many of them are partially dependent on the cell to do this. They therefore modify gene expression in the host cell to favour DNA synthesis and cell proliferation. Several DNA viruses have developed common pathways

* Corresponding author.
E-mail addresses: stalbot@ed.ac.uk (S.J. Talbot), d.crawford@ed.ac.uk (D.H. Crawford).

0959-8049/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2003.11.039

Table 1
Human viruses with oncogenic potential

Virus family	Virus	Human tumours	Non-malignant disease	Experimental tumours in animals
Papovaviridae	Human papillomavirus	Cervical cancer, Anogenital cancer, Skin cancer	Warts	
	BK	None	Not known	Multiple tumours in rodents
	JC	None	Progressive multifocal leucoencephal-opathy	Multiple tumours in rodents
Adenoviridae	Adenovirus	None	Gut and respiratory infections	Sarcomas, carcinomas in rodents
Herpesviridae	Epstein–Barr virus	Nasopharyngeal carcinoma, Burkitt's lymphoma, Immunoblastic lymphoma, Hodgkin's lymphoma	Infectious mononucleosis	Lymphoma in primates
	Kaposi's sarcoma-associated herpesvirus	Kaposi's sarcoma, Primary effusion lymphoma	Multicentric Castleman's disease	Not known
Retroviridae	HTLV-1	Adult T-cell Leukaemia (ATL)	Tropical spastic paraparesis	ATL in rabbits
	HIV (indirect)	B-cell lymphoma, Kaposi's sarcoma	AIDS	
Hepadnaviridae	Hepatitis B virus	Liver cancer	Hepatitis, Cirrhosis	Not known
Flaviviridae	Hepatitis C virus (indirect)	Liver cancer	Hepatitis, Cirrhosis	Not known

HTLV-1, human T-cell lymphotrophic virus type-1; HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome.

to circumvent the problem of replicating in quiescent or post-mitotic, terminally differentiated cells. Adenoviruses, polyoma virus and papillomavirus strains have the capacity to inactivate the same cellular tumour suppressor proteins, namely retinoblastoma (Rb) and p53. Blockade of Rb and p53 function removes the checkpoint that controls the cell cycle by arresting cells in G0–G1 and allows cells to proliferate indefinitely. p53 also maintains genome stability and may induce apoptosis, such that removal of its function would generate genomic instability leading to a higher probability of mutations and chromosomal rearrangements. Moreover, cells normally programmed to apoptotic cell death may survive and become prone to transformation.

The most recent member of the human herpesvirus family to be identified – Kaposi's sarcoma-associated herpesvirus (KSHV; human herpesvirus-8, HHV-8) – has also evolved mechanisms for the inactivation of the tumour suppressors p53 and Rb. The KSHV encoded latency associated nuclear antigen (LANA) is a versatile protein with multiple functions. It tethers viral episomes (circular DNA) to host chromatin during mitosis, allowing delivery of viral progeny to all daughter cells [10]. Similar to papillomavirus E6 and E7 and other oncogenes of DNA tumour viruses, LANA binds to and interferes with the functions of the tumour suppressors p53 and the retinoblastoma protein [11,12] and transforms primary rat embryo cells with *Hras* [12]. Recently, it was reported that LANA also exploits the Wnt-β-catenin pathway in an unusual way to activate genes that can promote cell growth [13]. This finding links KSHV oncogenesis to pathways critical to cell differentiation and pathways known to be deregulated in non-virally associated cancers. The multifunctional activities of LANA are reminiscent of the SV40 large T antigen and may contribute to the induction of tumours such as Kaposi's sarcoma and primary effusion lymphoma.

3. Retroviruses and oncogenes

Retroviruses are implicated in a series of human and animal tumours such as leukaemias, mammary tumours and skin cancer. The mechanisms that they use to induce tumour formation vary. Insertional mutagenesis is a common mechanism in rodent, feline and avian retroviruses, where the retrovirus integrates into the host genome and affects the transcription of the neighbouring genes. Cloning of these affected genes led to identification of a series of oncogenes that play a significant role in the induction of human neoplasms. Retrovirus insertion also serves as a model to identify collaborating oncogenes. Human retroviruses use different, more complex mechanisms contributing to oncogenesis. Studies of the propagation and induction mechanisms used by retroviruses have given insight to the understanding of oncogenesis.

At the time of publication of Harris' article, Temin [14] had just published his paper proposing that retroviruses somehow translated their RNA into DNA, which then redirected the reproductive activity of the cell, transforming it into a cancer cell. Sceptics pointed out that Temin's suggestion contradicted the contemporary tenet of molecular biology: that genetic

information always passed from DNA to RNA, rather than the reverse. But in 1970 both Temin and Baltimore proved Temin's hypothesis correct. They identified an enzyme (reverse transcriptase) in the virus that synthesises DNA that contains the information in the viral RNA [15,16], a discovery that was rewarded with a Nobel prize in 1975.

4. Leukaemia

In his article, Harris discusses the great interest in the observed clusters of leukaemia in children and the presence of virus-like particles in the tissues of some of these patients. Despite extensive research in the area, no virus or other infectious agent has been discovered as the cause of such leukaemia. Kinlen and Balkwill [17] have suggested that unusual mixing of rural and urban populations is associated with increased cases of childhood leukaemia, which would be predicted if this disease had an underlying infectious cause. In their study of the populations of the Orkney and Shetland islands during World War II, childhood leukaemia increased 3.6-fold in wartime, but not in the postwar period, compared with the national Scottish rate. The authors attribute this increase to the large influx of servicemen stationed there during the war, suggesting that these findings add to the evidence for infection as a cause of childhood leukaemia. A similar study of the cluster of childhood leukaemia at the nuclear reprocessing plant at Seascale, Cumbria, concluded that this might also be attributed to the influx of workers from urban areas to this rural location [18].

4.1. Adult T cell leukaemia and human T-lymphotrophic virus-1

The only virus found to be associated with human leukaemia is HTLV-1. HTLV-1, discovered after intense efforts to isolate retroviruses by Gallo [19], is associated with specific lymphoid malignancies and is endemic in Japan, the Caribbean and Africa. Molecular probes show that HTLV-1 is present in almost all cases of adult T cell leukaemia (ATL). There is also a strong sero-epidemiological correlation between the disease and the virus. Transmission is thought to occur by cell-cell contact of virus-infected cells during the intimate exchange of body fluids. Possible routes of transmission include, perinatal transmission via milk lymphocytes during breast feeding, lymphocytes contained in semen during sexual intercourse, unscreened blood transfusions and sharing of blood contaminated needles by intravenous drug users. The virus is becoming increasingly common in W. Europe and N. America particularly amongst intravenous drug users and homosexual men. Approximately 1–3% of infected individuals will eventually develop ATL after an incubation period that is usually several decades long. Following an asymptomatic period, the patient may develop pre-ATL, in which they remain asymptomatic, but there is a clonal expansion of T-cells that can be morphologically-altered. Half of patients with pre-ATL progress to chronic/smouldering ATL that is manifested by skin lesions (*mycosis fungoides*) and high leucocyte counts. Progression to acute ATL occurs within several months and is rapidly fatal.

The pathogenesis of HTLV-1 apparently results from the pleiotropic function of the Tax protein, which is a key regulator of viral replication. Tax has been shown to have multiple activities in T cells. Accumulating evidence indicates that activation of cellular genes through the transcription factor nuclear factor κB (NF-κB) by Tax is a critical factor for the transforming activity. For example, Tax activates the transcription of numerous cellular genes, such as genes encoding cytokines (interleukin-2 (IL-2) and IL-8), the cytokine receptors (α-chain of IL-2 receptor), proto-oncogenes (c-*fos*, c-*jun*, *fra-1*, and c-*rel*), chemokines (IL-8 and stromal cell-derived factor 1 (SDF-1)), the anti-apoptotic gene *bcl-xl*, and the cell cycle regulator cyclin D2. In addition, Tax inactivates several tumour suppressor gene products, such as p53, p16INK, and hDLG (Reviewed in [20,21]). These effects on a wide variety of cellular targets seem to cooperate in promoting cell proliferation. This is an effective viral strategy to amplify its proviral genome through replication of infected cells; ultimately, it results in an expanded T cell population in which a malignant event can occur.

4.2. Leukaemia caused by gene therapy vectors

Gene transfer to human cells is being considered as an approach to treating numerous congenital and acquired disorders. Recombinant viruses are, at present, the most effective vectors for transferring genes into primary cells. The use of oncoretroviruses and lentiviruses has been extensively investigated as vectors due to their ability to integrate into the host cell genome. Retroviral integration can occur in a large number of different locations on different chromosomes, although it seems that preferred sites are within or near genes that are transcriptionally active [22].

A recent gene therapy trial [23] tested the ability of a retroviral vector to treat patients who had a severe combined immunodeficiency (SCID), and resulted in restored immune function in most patients. Unfortunately, almost 3 years after therapy was completed, T-cell leukaemia developed in two of the subjects that were involved in the trial. In both cases, the leukaemia cells contained a single intact copy of the retroviral vector, which had integrated into chromosome 11, at or near the *LMO2* proto-oncogene [24]. *LMO2* is involved in the development of both the lymphoid and myeloid se-

ries and is the site of a translocation in leukaemia. *LMO2* transcription is activated in childhood acute lymphoblastic leukaemia (ALL) cells as a consequence of a t(11;14)(p13;q11) translocation [25]. These two cases of leukaemia raise some interesting questions. Is retroviral integration casually linked to leucaemogenesis? Is integration near *LMO2* unique for patients with X-linked SCID or a more frequent event? This serious adverse complication in a trial that initially produced promising results has brought similar clinical trials to a halt for the time being.

Long-term integration of gene sequences into a patient's genome is the very property that makes retroviruses attractive vectors for delivering genes to correct inherited mono-genic disorders. Only a few studies in animal models of gene therapy have suggested that vector-mediated insertional mutagenesis could be a problem [26].

5. Burkitt's lymphoma

In 1958, Denis Burkitt first described a jaw tumour common in African children that now bears his name [27], and in 1962, after finding that the geographical distribution of Burkitt's lymphoma (BL) closely mapped to that of holoendemic malaria, he postulated that it was caused by an infectious agent and transmitted by the mosquito [28]. It is clear from Harris's article that after publication of Burkitt's hypothesis several research groups attempted to isolate a virus from BL tissue, and it is interesting that Harris, writing a year after the isolation of EBV was reported in 1964 [29], mentions this new herpesvirus only in a list of reported virus isolations, including two other herpesviruses, herpes simplex virus and cytomegalovirus, now known to be non-tumorigenic and unrelated to BL. Clearly it took several years to establish an aetiological association between EBV and BL, the information accumulating only as research techniques became sophisticated enough to study virus/cell interactions at the molecular level.

BL is a malignancy of B lymphocytes, and in addition to endemic (African) BL which is almost entirely EBV-associated, sporadic cases of BL occur throughout the world at a low incidence which have a weaker EBV association. Furthermore, over the 40 years since Harris wrote his review, EBV has been associated with many other tumours (Table 1). However, this review will concentrate on endemic BL as described by Burkitt in 1962 [28].

Primary EBV infection is generally asymptomatic, and following this the virus persists as a latent infection of B-lymphocytes for life. Over 90% of the adult population worldwide carry EBV, and in BL endemic areas almost every child over the age of 2 years has evidence of persistent infection [30]. Thus, it rapidly became apparent that EBV infection alone is not sufficient to cause BL, thereby establishing the concept of a multi-factorial aetiology with EBV as one essential factor. Burkitt demonstrated a geographical association between BL and holoendemic malaria [28], and although his suggestion of mosquito transmission proved false (the virus is transmitted by salivary contact), the link with malaria infection remains valid, albeit not fully explained.

Evidence for the tumorigenic nature of EBV comes from its ability to cause B cell tumours on inoculation into certain sub-human primates [31], and to immortalise B lymphocytes after in vitro infection yielding lymphoblastoid cell lines (LCLs) [32]. Analysis of LCLs has been invaluable in understanding the complex virus/cell interactions that initiate and maintain B cell growth, and once the EBV genome had been sequenced [33] genes could be mapped and their transcription patterns elucidated.

A unique set of latent viral genes is expressed in LCLs that act in concert to drive B cell proliferation. Of prime importance in this regard are two viral oncogenes, EB viral nuclear antigen (EBNA)2 and latent membrane protein (LMP)1. EBNA2 is a functional homologue of Notch and transactivates the other latent viral genes and cellular genes such as *c-myc* and *c-fgr* to induce B cell proliferation and inhibit differentiation (for a review see [34,35]). LMP1 acts as a constitutively active tumour necrosis factor (TNF)-receptor family member inducing B cell activation and proliferation via induction of NF-kB (for a review see [34,35]).

Examination of BL cells shows a clonal, germinal centre B cell phenotype [36] with every cell containing multiple circular episomes of EBV DNA. The viral DNA is also clonal [37] indicating that the infection event occurs before expansion of the tumorigenic B cell clone and thus implicating the virus in tumour initiation and outgrowth. Analysis of EBV latent gene expression in BL tumour cells reveals a very restricted pattern compared with LCLs. The EBNA1 protein, which is essential for maintenance of the viral genome [38], and the EBV-encoded small RNAs (EBERs) are expressed in the absence of EBNA2 and LMP1 [39]. No oncogenic role has been identified for EBNA1 in BL, although recent experimental data suggests that the EBERs can enhance cell survival and proliferation [40]. The lack of EBNA2 expression in BL, caused in a subset of tumours by gene deletion [41], abrogates the transactivation of cellular genes that are essential to induce cell proliferation in LCLs. However, this is compensated for by constitutive expression of the *c-myc* oncogene found in all BL tumours. The *c-myc* oncogene situated on chromosome 8 induces cell proliferation and inhibits differentiation. It has long been recognised that an 8:14 chromosomal translocation (or more rarely 8:2 or 8:22) is invariably found in BL cells whether EBV-associated

or not [42]. This translocation brings *c-myc* under the control of the immunoglobulin heavy chain (on chromosome 14) or light chain (on chromosomes 2 and 22) genes and induces constitutive expression. Other genetic abnormalities found in BL include p53 mutations that are thought to occur late in tumour progression [43]. Thus, although the pathogenesis of BL has not yet been fully elucidated, many steps in the tumorigenic process have been identified.

6. Tumour antigens

Harris discusses tumour antigens in detail, distinguishing between complement-fixing and transplantation antigens that he considers to be virus-specific, and non-virion neo-antigens which were thought to be soluble viral products, possibly enzymes. He pointed out that it was relatively easy to search for viruses by staining tumour tissue with sera from infected animals, but there were difficulties in the interpretation of these results. This was mainly due to the polyclonal nature of the antibodies used prior to 1975 when monoclonal antibodies became available and these problems were overcome by the exquisite specificity of the new reagents [44].

Most cells in viral-induced tumours are not productively infected, but express a limited set of viral genes that induce cell proliferation. The characterisation of these viral antigens and the definition of the immune response directed against them have proceeded hand in hand, and, in many cases, we now have a clear picture of the major antigenic determinants and immunodominant epitopes.

It is now clear that most tumour viruses establish persistent infections years before the malignancy arises, and that this is mainly controlled by classical human leucocyte antigen (HLA) class 1 restricted, CD8-positive cytotoxic T cells (CTL). In healthy individuals, CTL eliminate cells expressing viral antigens and prevent tumour outgrowth. Thus, for a tumour to develop other events, such as integration of the viral genome, must occur. One such event is immunosuppression since in those with congenital, acquired or iatrogenic immunosuppression, CTL activity is often defective and, consequently, virus-associated tumours are much more common than in the general population [45].

Recently, the idea of harnessing the immune response to prevent or treat tumours has become a reality since CTL can be cultured from the peripheral blood of virus-infected individuals and induced to become specific for a particular antigen by repeated rounds of antigenic stimulation. This approach is particularly apt for virus-associated tumours where the target antigens are expressed uniquely on tumour cells and their identity is known. T cell immunotherapy has been pioneered for EBV-associated post-transplant lymphoproliferative disease (PTLD) (reviewed in [46]) that develops in up to 10% of transplant recipients as a result of the immunosuppressive drugs required to prevent graft rejection (reviewed in [47]). PTLD cells generally express all the EBV latent viral genes, of which EBNA 3a,b,c are known to be immunodominant. To date, autologous CTL grown from bone marrow donors have been used to prevent and treat PTLD in the bone marrow recipient [48,49]. However, this procedure is expensive and time-consuming as each CTL line has to be tailor-made for each recipient. Furthermore, this approach cannot be used for PTLD in solid organ transplant recipients where the donor is either not available or not HLA compatible. Another approach is to derive a frozen bank of CTL from healthy donors covering all common HLA types and use these on a best HLA match basis. This has been used in a phase 1/2 clinical trial with encouraging results [50] and a larger randomised controlled trial is now underway.

7. Identification of tumour-associated viruses through the use of modern molecular techniques

Harris writes about the difficulties encountered in the isolation of viruses from tumours and the subsequent burden of proof required to show that the isolated virus is the cause of the tumour rather than simply a passenger. The advent of modern techniques in molecular biology has enabled the isolation of human tumour viruses, as well as speeding up epidemiological studies through the use of polymerase chain reaction (PCR) screening.

7.1. Kaposi's sarcoma-associated herpesvirus

Kaposi's sarcoma (KS) was first described as 'idiopathic multiple pigmented sarcomas of the skin' by the Hungarian dermatologist Moritz Kaposi in 1872 [51]. Strong epidemiological evidence accumulated since the 1980s suggested that KS was caused by an infectious agent [52]; KS is 200 times more common in acquired immunodeficiency syndrome (AIDS) patients than in other immunosuppressed groups, and 10 times more common in homosexual or bisexual men with AIDS than in groups of HIV-infected individuals who have become infected by non-sexual routes. KS is now recognised as the leading neoplasm of AIDS patients, and epidemiological evidence suggests that a sexually transmitted factor other than HIV plays a key role in its development.

In 1994, Chang and Moore [53] used the technique of representational difference analysis (RDA) to isolate unique DNA sequences present in more than 90 percent of KS tissues obtained from patients with AIDS. When sequenced, the DNA fragments were found to be homologous to, but distinct from, capsid and tegument protein genes of the Gamma herpesvirinae, herpesvirus

Saimiri and EBV. The newly identified KS-associated herpesvirus (KSHV) has since been implicated in the aetiology of all epidemiological forms of KS, i.e. Mediterranean classic, African endemic, post-transplant or iatrogenic and AIDS-associated. KSHV sequences have also been identified in several rare lymphomas such as primary effusion lymphoma (PEL), also known as body cavity-based large-cell lymphoma.

The technique used to identify KSHV (RDA) was first described by Lisitsyn [54]. It is a procedure that analyses the difference between two complex sets of genomes. By comparing DNA from diseased and normal cells from a sample, the method can identify DNA sequences that differ between diseased and normal cells. In addition, it can assess genetic changes that occur during tumour development and detect deletions, rearrangements, or extraneous viral DNA sequences. RDA enriches for unique DNA sequences from one of the genomes by removing shared sequences and it uses PCR to amplify those distinct regions.

7.2. Hepatitis C virus

As with HBV, chronic HCV infection is a major risk factor for HCC (30% of United Kingdom (UK) HCC patients). Time from HCV transmission to development of cancer ranges from 10 to 50 years (median 30 years). There is a strong association between chronic HCV infection, cirrhosis and HCC.

Originally one source of non-A non-B hepatitis (NANBH), HCV was first definitively identified by molecular cloning of the virus genome in 1989 [55]. A random-primed complementary DNA library was constructed from plasma containing the uncharacterised NANBH agent and screened with serum from a patient diagnosed with NANBH. A complementary DNA clone was isolated that was shown to encode an antigen associated specifically with NANBH infections. The clone was derived from an RNA molecule present in NANBH infections with sequence homology with members of the flaviviridae.

Since so little is known about the biology of HCV, it is presently unclear how this RNA virus establishes a persistent infection. However, it is known that there is a very rapid turnover of plasma virus in patients, with particle half-lives of 100–182 min. Recently, it has been suggested that subversion of the humoral immune response, specifically neutralising antibody production, may allow HCV to persist. It appears that in most HCV-infected patients, the development of antibody exerts immune pressure that increases viral diversity and leads to an increasingly complex population that can elude the immune attack and result in viral persistence [56].

The HCV core protein has been shown to interact with p53 and modulate p53-dependent promoter activities during HCV infection [57]. Together with the damage caused to the liver by chronic HCV infection, this may play a role in the survival of tumour cells transformed by HCV.

8. The future

Despite the many discoveries and advances over the last 40 years, there still remains several unanswered questions regarding the viral aetiology of cancer.

Immunosuppression through infection with HIV or iatrogenic means causes a marked increase in the incidence of non-melanoma skin cancer and some virally-induced cancers [58]. The discovery that many other epithelial cancers, notably lung, colon, rectum, bladder and prostate (but not breast), are also increased by immunosuppression suggests that unidentified viruses may be important in these cancers as well [59].

The possibility that viruses related to the Mouse mammary tumour virus (MMTV) can cause some breast cancers has been investigated since at least 1970. The evidence has been contradictory with some studies finding up to 40% of breast cancer tissues containing sequences that are 95% identical to MMTV [60]. This virus has been termed HMTV (Human mammary tumour virus). However, other studies have failed to find any evidence for such a link [61].

While the role of different viruses in the aetiology of human cancers varies widely and their scope as treatment targets remains unclear, few would dispute that more viruses will be found to be associated with cancers in the coming years.

References

1. Harris RJ. Viruses and tumours. *Eur J Cancer* 1965, **1**, 183–188.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001, **94**, 153–156.
3. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, *et al*. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999, **189**, 12–19.
4. IARC. Human Papillomaviruses (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans) 64, 1995, Lyon.
5. IARC. *Hepatitis Viruses* (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans) 59, 1994, Lyon.
6. IARC. Human Immunodeficiency Viruses and Human T-cell Lymphotropic Viruses (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans) 67, 1996, Lyon.
7. IARC. Epstein–Barr Virus and Kaposi's Sarcoma Herpesvirus/Human Herpesvirus 8 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans) 70, 1997, Lyon.
8. Kinlen LJ. Infection and childhood leukemia. *Cancer Causes Control* 1998, **9**, 237–239.
9. Tjian R, Robbins A. Enzymatic activities associated with a purified simian virus40 T antigen-related protein. *Proc Natl Acad Sci USA* 1979, **76**, 610–614.
10. Ballestas ME, Chatis PA, Kaye KM. Efficient persistence of extrachromosomal KSHV DNA mediated by latency-associated nuclear antigen. *Science* 1999, **284**, 641–644.

11. Friborg Jr J, Kong W, Hottiger MO, Nabel GJ. p53 inhibition by the LANA protein of KSHV protects against cell death. *Nature* 1999, **402**, 889–894.
12. Radkov SA, Kellam P, Boshoff C. The latent nuclear antigen of Kaposi sarcoma-associated herpesvirus targets the retinoblastoma-E2F pathway and with the oncogene Hras transforms primary rat cells. *Nat Med* 2000, **6**, 1121–1127.
13. Fujimuro M, Wu FY, ApRhys C, Kajumbula H, Young DB, Hayward GS, *et al*. A novel viral mechanism for dysregulation of beta-catenin in Kaposi's sarcoma-associated herpesvirus latency. *Nat Med* 2003, **9**, 300–306.
14. Temin HM. Genetic and possible biochemical mechanisms in viral carcinogenesis. *Cancer Res* 1966, **26**, 212–216.
15. Baltimore D. RNA-dependent DNA polymerase in virions of RNA tumour viruses. *Nature* 1970, **226**, 1209–1211.
16. Temin HM, Mizutani S. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. *Nature* 1970, **226**, 1211–1213.
17. Kinlen LJ, Balkwill A. Infective cause of childhood leukaemia and wartime population mixing in Orkney and Shetland, UK. *Lancet* 2001, **357**, 858.
18. Kinlen LJ, Dickson M, Stiller CA. Childhood leukaemia and non-Hodgkin's lymphoma near large rural construction sites, with a comparison with Sellafield nuclear site. *BMJ* 1995, **310**, 763–768.
19. 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 USA* 1980, **77**, 7415–7419.
20. Mortreux F, Gabet AS, Wattel E. Molecular and cellular aspects of HTLV-1 associated leukemogenesis in vivo. *Leukemia* 2003, **17**, 26–38.
21. Yoshida M. Multiple viral strategies of HTLV-1 for dysregulation of cell growth control. *Annu Rev Immunol* 2001, **19**, 475–496.
22. Vijaya S, Steffen DL, Robinson HL. Acceptor sites for retroviral integrations map near DNase I-hypersensitive sites in chromatin. *J Virol* 1986, **60**, 683–692.
23. Cavazzana-Calvo M, Hacein-Bey S, de Saint BG, Gross F, Yvon E, Nusbaum P, *et al*. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 2000, **288**, 669–672.
24. Kohn DB, Sadelain M, Glorioso JC. Occurrence of leukaemia following gene therapy of X-linked SCID. *Nat Rev Cancer* 2003, **3**, 477–488.
25. Royer-Pokora B, Loos U, Ludwig WD. TTG-2, a new gene encoding a cysteine-rich protein with the LIM motif, is overexpressed in acute T-cell leukaemia with the t(11;14)p(13;q11). *Oncogene* 1991, **6**, 1887–1893.
26. Li Z, Dullmann J, Schiedlmeier B, Schmidt M, von Kalle C, Meyer J, *et al*. Murine leukemia induced by retroviral gene marking. *Science* 2002, **296**, 497.
27. Burkitt D. A sarcoma involving the jaws of African children. *Brit J Surg* 1958, **46**, 218–223.
28. Burkitt D. Determining the climatic limitations of a childrens cancer common in Africa. *Brit Med J* 1962, **2**, 1019–1023.
29. Epstein MA, Barr YM, Achong BG. Virus particles in cultured lymphoblasts from Burkitts lymphoma. *Lancet* 1964, **1**, 702–803.
30. de The G, Geser A, Day NE, Tukei PM, Williams EH, Beri DP, *et al*. Epidemiological evidence for causal relationship between Epstein–Barr virus and Burkitt's lymphoma from Ugandan prospective study. *Nature* 1978, **274**, 756–761.
31. Deinhardt F, Deinhardt J. Comparative aspects: oncogenic animal herpesviruses. In Epstein MA, Achong BG, eds. *The Epstein–Barr virus*. Berlin, Heidelberg, New York, Springer-Verlag, 1979, pp 373–415.
32. Pope JH, Horne MK, Scott W. Transformation of foetal human keukocytes in vitro by filtrates of a human leukaemic cell line containing herpes-like virus. *Int J Cancer* 1968, **3**, 857–866.
33. Baer R, Bankier AT, Biggin MD, Deininger PL, Farrell PJ, Gibson TJ, *et al*. DNA sequence and expression of the B95-8 Epstein–Barr virus genome. *Nature* 1984, **310**, 207–211.
34. Bornkamm GW, Hammerschmidt W. Molecular virology of Epstein–Barr virus. *Philos Trans R Soc Lond B: Biol Sci* 2001, **356**, 437–459.
35. Young LS, Dawson CW, Eliopoulos AG. The expression and function of Epstein–Barr virus encoded latent genes. *Mol Pathol* 2000, **53**, 238–247.
36. Gregory CD, Edwards CF, Milner A, Wiels J, Lipinski M, Rowe M, *et al*. Isolation of a normal B cell subset with a Burkitt-like phenotype and transformation in vitro with Epstein–Barr virus. *Int J Cancer* 1988, **42**, 213–220.
37. Raab-Traub N, Flynn K. The structure of the termini of the Epstein–Barr virus as a marker of clonal cellular proliferation. *Cell* 1986, **47**, 883–889.
38. Yates JL, Warren N, Sugden B. Stable replication of plasmids derived from Epstein–Barr virus in various mammalian cells. *Nature* 1985, **313**, 812–815.
39. Rowe M, Rowe DT, Gregory CD, Young LS, Farrell PJ, Rupani H, *et al*. Differences in B cell growth phenotype reflect novel patterns of Epstein–Barr virus latent gene expression in Burkitt's lymphoma cells. *EMBO J* 1987, **6**, 2743–2751.
40. Komano J, Maruo S, Kurozumi K, Oda T, Takada K. Oncogenic role of Epstein–Barr virus-encoded RNAs in Burkitt's lymphoma cell line Akata. *J Virol* 1999, **73**, 9827–9831.
41. Kelly G, Bell A, Rickinson A. Epstein–Barr virus-associated Burkitt lymphomagenesis selects for downregulation of the nuclear antigen EBNA2. *Nat Med* 2002, **8**, 1098–1104.
42. Zech L, Haglund U, Nilsson K, Klein G. Characteristic chromosomal abnormalities in biopsies and lymphoid-cell lines from patients with Burkitt and non-Burkitt lymphomas. *Int J Cancer* 1976, **17**, 47–56.
43. Farrell PJ, Allan GJ, Shanahan F, Vousden KH, Crook T. p53 is frequently mutated in Burkitt's lymphoma cell lines. *EMBO J* 1991, **10**, 2879–2887.
44. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975, **256**, 495–497.
45. Penn I. The changing pattern of posttransplant malignancies. *Transplant Proc* 1991, **23**, 1101–1103.
46. Haque T, Crawford DH. The role of adoptive immunotherapy in the prevention and treatment of lymphoproliferative disease following transplantation. *Br J Haematol* 1999, **106**, 309–316.
47. Hopwood P, Crawford DH. The role of EBV in post-transplant malignancies: a review. *J Clin Pathol* 2000, **53**, 248–254.
48. Heslop HE, Ng CY, Li C, Smith CA, Loftin SK, Krance RA, *et al*. Long-term restoration of immunity against Epstein–Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. *Nat Med* 1996, **2**, 551–555.
49. Rooney CM, Smith CA, Ng CY, Loftin S, Li C, Krance RA, *et al*. Use of gene-modified virus-specific T lymphocytes to control Epstein–Barr-virus-related lymphoproliferation. *Lancet* 1995, **345**, 9–13.
50. Haque T, Wilkie GM, Taylor C, Amlot PL, Murad P, Iley A, *et al*. Treatment of Epstein–Barr-virus-positive post-transplantation lymphoproliferative disease with partly HLA-matched allogeneic cytotoxic T cells. *Lancet* 2002, **360**, 436–442.
51. Kaposi M. *Arch Dermatol Syphillis* 1872, **4**, 265–273.
52. Beral V, Newton R. Overview of the epidemiology of immunodeficiency-associated cancers. *J Natl Cancer Inst Monogr* 1998, 1–6.
53. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, *et al*. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 1994, **266**, 1865–1869.
54. Lisitsyn N, Lisitsyn N, Wigler M. Cloning the differences between two complex genomes. *Science* 1993, **259**, 946–951.
55. 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**, 359–362.

56. Farci P, Shimoda A, Coiana A, Diaz G, Peddis G, Melpolder JC, *et al*. The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. *Science* 2000, **288**, 339–344.
57. Otsuka M, Kato N, Lan K, Yoshida H, Kato J, Goto T, *et al*. Hepatitis C virus core protein enhances p53 function through augmentation of DNA binding affinity and transcriptional ability. *J Biol Chem* 2000, **275**, 34122–34130.
58. Kinlen LJ, Sheil AG, Peto J, Doll R. Collaborative United Kingdom–Australasian study of cancer in patients treated with immunosuppressive drugs. *Br Med J* 1979, **2**, 1461–1466.
59. Birkeland SA, Storm HH, Lamm LU, Barlow L, Blohme I, Forsberg B, *et al*. Cancer risk after renal transplantation in the Nordic countries, 1964–1986. *Int J Cancer* 1995, **60**, 183–189.
60. Ford CE, Tran D, Deng Y, Ta VT, Rawlinson WD, Lawson JS. Mouse mammary tumor virus-like gene sequences in breast tumors of Australian and Vietnamese women. *Clin Cancer Res* 2003, **9**, 1118–1120.
61. Witt A, Hartmann B, Marton E, Zeillinger R, Schreiber M, Kubista E. The mouse mammary tumor virus-like env gene sequence is not detectable in breast cancer tissue of Austrian patients. *Oncol Rep* 2003, **10**, 1025–1029.