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The role of Epstein-Barr virus infection in human malignancies

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The geographical distribution of Burkitt's lymphoma (BL), a common cancer affecting children in equatorial Africa originally described by Denis Burkitt in 1958, was found to be dependent on climatic and geographical conditions leading to the suggestion that a vector-borne virus might be responsible (Burkitt, 1958; Burkitt, 1962). In 1964 Epstein, Achong and Barr identified herpesvirus-like particles by electron microscopy in a cell line established from a BL biopsy (Epstein *et al*., 1964). In the late 1960s it was shown that sera from BL patients had higher antibody titres to Epstein-Barr virus (EBV) antigens than controls (Henle and Henle, 1966). These serological assays also identified EBV as the aetiological agent of infectious mononucleosis (IM) and resulted in the demonstration that EBV infection was a common feature of another malignancy, undifferentiated nasopharyngeal carcinoma (NPC) (Henle *et al.,* 1968; zur Hausen *et al.,* 1970). At about the same time, the transformation capacity of EBV was confirmed by the ability of the virus to efficiently transform resting B cells *in vitro* and induce tumours in non-human primates (Henle *et al.,* 1967; Pope *et al.,* 1968; Miller, 1974). Subsequent studies identified an association between EBV infection and a variety of other human tumours including B cell malignancies such as Hodgkin's lymphoma (HL) and lymphoproliferative disease arising in immunosuppressed patients, some T cell lymphomas, and epithelial tumours such as gastric cancer. All of these tumours are characterised by the presence of multiple extrachromosomal copies of the circular viral genome in the tumour cells and expression of the EBV-encoded latent genes, which appear to contribute to the malignant phenotype (Rickinson and Kieff, 2001).

The virus and its natural history of infection

EBV is a gammaherpesvirus of the *Lymphocryptovirus* (LCV) genus and is closely related to other LCVs present in Old World non-human primates, including EBV-like viruses of chimpanzees and rhesus monkeys. The EBV genome is composed of linear double-stranded DNA, approximately 172 kilobase pairs (kb) in length. EBV has a series of 0.5 kb terminal direct repeats (TRs) and internal repeat sequences (IRs) that divide the genome into short and long, largely unique sequence domains. EBV was the first herpesvirus to have its genome completely cloned and sequenced (Baer *et al.,* 1984). Since the EBV genome was sequenced from an EBV DNA *Bam*HI fragment cloned library, open reading frames (ORFs), genes and sites for transcription or RNA processing are frequently referenced to specific *Bam*HI fragments, from A to Z, in descending order of fragment size.

EBV infects the majority of the World's adult population and following primary infection the individual remains a lifelong carrier of the virus. In underdeveloped countries, primary infection with EBV usually occurs during the first few years of life and is often asymptomatic. However, in developed populations, primary infection is more frequently delayed until adolescence or adulthood, in many cases producing the characteristic clinical features of IM. EBV is orally transmitted, and infectious virus can be detected in oropharyngeal secretions from IM patients, from immunosuppressed patients and at lower levels from healthy EBV seropositive individuals (Gerber *et al.,* 1972; Strauch *et al.,* 1974; Yao *et al.,* 1985). Early in the course of primary infection, EBV infects B lymphocytes, although it is not known where B lymphocytes are infected and whether this involves epithelial cells. EBV does not usually replicate in B lymphocytes but instead establishes a latent infection, which is characterised by the limited expression of a subset of virus latent genes. Recent work highlights the role of these EBV genes in the colonisation of the B cell pool and the establishment of persistent infection (Thorley-Lawson, 2001). These studies also emphasise that the inappropriate expression of EBV latent genes involved in B cell persistence can have dangerous conse-

quences by contributing to the pathogenesis of virus-associated tumours.

The lymphoblastoid cell line – an in vitro model of EBV infection and transformation

When peripheral blood lymphocytes from chronic virus carriers are placed in culture, the few EBV-infected B cells that are present regularly give rise to spontaneous outgrowth of EBV-transformed cell lines, known as lymphoblastoid cell lines (LCLs), provided that immune T cells are either removed or inhibited by addition of cyclosporin A to the culture (Rickinson *et al.,* 1984). LCLs can also be generated by infecting resting B cells with EBV and this has proved to be an invaluable tool in isolating and perpetuating the genotype of individuals for genetic and other studies. Every cell in an LCL carries multiple copies of the viral episome and constitutively expresses a limited set of viral gene products, the so-called latent proteins, consisting of six nuclear antigens (EBNAs 1, 2, 3A, 3B, 3C and -LP) and three latent membrane proteins (LMPs 1, 2A and 2B) (Kieff and Rickinson, 2001). Transcripts from the *Bam*HI A region of the viral genome (so-called BART transcripts), first identified in NPC cells, are also detected in LCLs though the ability of these mRNAs to encode proteins remains controversial (Brooks *et al.,* 1992). In addition to the latent proteins, LCLs also show abundant expression of the small non-polyadenylated (and therefore non-coding) RNAs, EBERs 1 and 2; the function of these transcripts is not clear but they are consistently expressed in all forms of latent EBV infection (Kieff and Rickinson, 2001). The different EBNAs are encoded by individual mRNAs generated by differential splicing of the same long 'rightward' primary transcript expressed from one of two promoters (Cp and Wp) located close together in the *Bam*HI C and W region of the genome (Speck and Strominger, 1989). The LMP transcripts are expressed from separate promoters in the *Bam*HI N region of the EBV genome, with the leftward LMP1 and rightward LMP2B mRNAs apparently controlled by the same bi-directional promoter sequence (Speck and Strominger, 1989; Rickinson and Kieff, 2001). This pattern of latent EBV gene expression is referred to as the "latency III" (Lat III) form of EBV infection.

LCLs show high level expression of the B cell activation markers CD23, CD30, CD39 and CD70 and of the cellular adhesion molecules LFA1 (CD11a/18), LFA3 (CD58) and ICAM1 (CD54) (Rowe *et al.,* 1987). These markers are usually absent or expressed at low levels on resting B cells, but are transiently induced to high levels when these cells are activated into short-term growth by antigenic or mitogenic stimulation, suggesting that EBV-induced immortalisation can be elicited through the constitutive activation of the same cellular pathways that drive physiological B cell proliferation. The ability of EBNA2, EBNA3C and LMP1 to induce LCL-like phenotypic changes when expressed individually in human B cell lines implicates these viral proteins as key effectors of the immortalisation process (Wang *et al.,* 1990). Although the majority of LCLs are tightly latent, some contain a small proportion of cells in the lytic cycle. The switch from latency to the lytic cycle is mediated by expression of the BZLF1 and BRLF1 viral transactivator proteins, which in turn trigger a cascade of events, including the sequential expression of numerous 'early' and 'late' viral genes, and a concomitant down-regulation of some latent genes, culminating in cell death and release of infectious virions. Of the lytic cycle genes, the BCRF1 and BHRF1 genes are particularly interesting since they encode homologues of human genes. The BCRF1 gene is expressed late in the lytic cycle and encodes a protein with significant homology to human IL-10. The BCRF1 product is thought to down-regulate cytotoxic immune responses during virus replication (Suzuki *et al.,* 1995). BHRF1, also expressed to high levels during the lytic cycle, encodes a BCL-2 like protein and thus is likely to protect cells replicating EBV from apoptosis (Young *et al.,* 1999).

Ebv latent gene function

The use of recombinant EBV lacking individual latent genes has confirmed the absolute requirement for EBNA2 and LMP1 in the *in vitro* transformation of B cells and has highlighted a critical role for EBNA-LP, EBNA3A and EBNA3C in this process (Kieff and Rickinson, 2001). However, with the demonstration of more restricted patterns of EBV gene expression in tumours, the function of the latent genes has been the focus of much interest.

The EBV-encoded nuclear antigens or EBNAs

EBV-infected cells express a group of nuclear proteins that influence both viral and cellular gene transcription. The EBNA1 protein is expressed in all virus infected cells where its role in the maintenance and replication of the episomal EBV genome is achieved through sequence-specific binding to the plasmid origin of viral replication, oriP (Kieff and Rickinson, 2001). EBNA1 can also interact with certain viral promoters thereby contributing to the transcriptional regulation of the EBNAs (including EBNA1 itself) and of LMP1. The EBNA1 protein is separated into amino and carboxy terminal domains by a glycine-glycine-alanine (gly-ala) repeat sequence, whose main function appears to be to stabilise the mature protein from proteasomal breakdown rather than to act in its originally perceived role as an immune evasion domain (Levitskaya *et al.,* 1995; Lee *et al.,* 2004; Tellam *et al.,* 2004; Voo *et al.,* 2004). Gene knockout studies suggest that EBNA1 does not have a critical role in *in vitro* B cell transformation beyond viral

genome maintenance (Humme *et al.,* 2003). However, a more direct involvement in oncogenesis is suggested by the ability of B cell-directed EBNA1 expression to produce B cell lymphomas in transgenic mice (Wilson *et al.,* 1996) and by its possible contribution to BL cell survival *in vitro* (Kennedy *et al.,* 2003).

The inability of an EBV strain, P3HR-1, carrying a deletion of the gene encoding EBNA2 and the last two exons of EBNA-LP, to transform B cells *in vitro* was the first indication of the crucial role of the EBNA2 protein in the transformation process (Kieff and Rickinson 2001). Restoration of the EBNA2 gene into P3HR-1 has unequivocally confirmed the importance of EBNA2 in B cell transformation and has allowed the functionally relevant domains of the EBNA2 protein to be identified (Cohen *et al.,* 1989; Hammerschmidt and Sugden, 1989). EBNA2 interacts with a sequence-specific DNA binding protein, RBP-Jk, to transcriptionally activate cellular genes such as CD23 and the key LMP1 and LMP2A (Grossman *et al.,* 1994; Hsieh and Hayward, 1995; Kieff and Rickinson, 2001). EBNA-LP interacts with EBNA2 and is required for the efficient outgrowth of virus-transformed B cells *in vitro* (Mannick *et al.,* 1991; Sinclair *et al.,* 1994). The transcriptional activation mediated by EBNA2 in conjunction with EBNA-LP is modulated by the EBNA3 family of proteins which repress transactivation (Robertson *et al.,* 1996; Zhao *et al.,* 1996). An essential role for EBNA3A and EBNA3C in B cell transformation *in vitro* has been demonstrated using EBV recombinants (Tomkinson *et al.,* 1993). EBNA3C can co-operate with ras in rodent fibroblast transformation assays and disrupt cell cycle checkpoints (Parker *et al.,* 1996; Parket *et al.,* 2000). These effects are partly explained by the interaction of EBNA3C with factors which modulate gene transcription (e.g. histone deacetylase 1 (HDAC1), Nm23-H1, CtBP) or influence cell cycle progression (e.g. cyclin A) (Radkov *et al.,* 1999).

The EBV-encoded latent membrane proteins, LMP1 and LMP2

LMP1 is the major transforming protein of EBV behaving as a classical oncogene in rodent fibroblast transformation assays and being essential for EBV-induced B cell transformation *in vitro* (Wang *et al.,* 1985; Kaye *et al.,* 1993). LMP1 has pleiotropic effects when expressed in cells resulting in induction of cell surface adhesion molecules and activation antigens (Wang *et al.,* 1990), up-regulation of anti-apoptotic proteins (Bcl-2, A20) (Henderson *et al.,* 1991; Laherty *et al.,* 1992) and stimulation of cytokine production (IL-6, IL-8) (Eliopoulos *et al.,* 1997; Eliopoulos *et al.,* 1999). LMP1 functions as a constitutively activated member of the tumour necrosis factor receptor (TNFR) superfamily activating a number of signalling pathways in a ligand-independent manner (Mosialos, 1995; Gires *et al.,* 1997; Kilger *et al.,* 1998). Functionally, LMP1 resembles CD40, a member of the TNFR superfamily and can partially substitute for CD40 in vivo providing both growth and differentiation signals to B cells (Uchida *et al.,* 1999). The LMP1 protein activates a number of downstream signalling pathways that contributes to the many phenotypic consequences of LMP1 expression including the induction of various anti-apoptotic and cytokine genes (Eliopoulos and Young, 2001).

The two proteins encoded by the LMP2, LMP2A and LMP2B, are not essential for EBV-induced B cell transformation *in vitro* (Longnecker, 2000). However, expression of LMP2A in the B cells of transgenic mice abrogates normal B cell development allowing immunoglobulin (Ig)-negative cells to colonise peripheral lymphoid organs (Caldwell *et al.,* 1998). This suggests that LMP2A can drive the proliferation and survival of B cells in the absence of signalling through the B cell receptor (BCR). LMP2A can transform epithelial cells and enhance their adhesion and motility, effects which may be mediated by activation of the PI3-kinase/Akt pathway (Scholle *et al.,* 2000). Repressive effects of LMP2A expression have recently been reported in human and murine B cells and many of these target B cell-specific factors resulting in a phenotype similar to that of the malignant Hodgkin's and Reed Sternberg (HRS) cells in Hodgkin's Lymphoma (HL) and of germinal centre B cells (Portis *et al.,* 2003a; Portis *et al.,* 2003b). Alongside these effects, LMP2A was also found to induce expression of a range of genes involved in cell cycle induction, apoptosis inhibition and suppression of cell-mediated immunity. A recent study demonstrates that LMP2A can negatively regulate LMP1 expression as well as down-regulating the NF-κB and STAT transcription factor pathways (Stewart *et al.,* 2004).

Other EBV-encoded transcripts expressed during latent infection

In addition to the latent proteins, two small non-polyadenylated (non-coding) RNAs, EBERs 1 and 2 are expressed in all forms of latency. However, the EBERs are not essential for EBV-induced transformation of primary B lymphocytes (Kieff and Rickinson, 2001). The EBERs assemble into stable ribonucleoprotein particles with the auto-antigen La, with ribosomal protein L22 and bind the interferon-inducible, double-stranded RNA-activated protein kinase PKR (Takada and Nanbo, 2001). PKR has a role in mediating the antiviral effects of the interferons and it has been suggested that EBER-mediated inhibition of PKR function could be important for viral persistence (Nanbo *et al.,* 2002). Expression of EBERs in BL cell lines has been found to enhance tumorigenicity, promote cell survival and induce IL-10 expression (Ruf *et al.,* 2000; Takada and Nanbo, 2001). Such studies suggest that EBV genes previously shown

to be dispensable for transformation in B cell systems might make more important contributions to the pathogenesis of some EBV-associated malignancies and to EBV persistence than was previously appreciated.

A group of abundantly expressed RNAs encoded by the *Bam*HIA region of the EBV genome were originally identified in NPC but subsequently found to be expressed in other EBV-associated malignancies such as BL, HL and nasal T cell lymphoma as well as in the peripheral blood of healthy individuals (Hitt *et al.,* 1989; Chen *et al.,* 1999; Deacon *et al.,* 1993). These highly spliced transcripts are commonly referred to as either BARTs (*Bam*HIA rightward transcripts) or complementary strand transcripts (CSTs) (Karran *et al.,* 1992; Smith *et al.,* 2000). The protein products of these ORFs remain to be conclusively identified. Another transcript generated from the *Bam*HIA region is BARF1which encodes a 31kDa protein originally identified as an early antigen expressed upon induction of the EBV lytic cycle. Recent studies have demonstrated that BARF1 is a secreted protein which is expressed as a latent protein in EBV-associated NPC and gastric carcinoma (Decaussin *et al.,* 2000; zur Hausen *et al.,* 2000). BARF1 shares limited homology with the human CSF-1 receptor (*c-fms* oncogene) and displays oncogenic activity when expressed in rodent fibroblasts and simian primary epithelial cells (Sheng *et al.,* 2001).

Ebv-associated tumours

Lymphoproliferative disease in immunosuppression

The lymphoproliferations that arise following iatrogenic immunosuppression for transplant surgery are collectively known as post-transplant lymphoproliferative disorders (PTLDs). Similar tumours are observed in patients with certain forms of inherited immunodeficiency syndromes, such as X-linked lymphoproliferative syndrome and Wiscott-Aldrich syndrome, and in AIDS patients. They are most often of B cell origin and represent a family of lesions ranging from atypical polyclonal B cell proliferations, which often regress following withdrawal or reduction of immune suppression, to aggressive monomorphic non-Hodgkin's lymphomas (NHLs), which generally do not resolve following immune reconstitution (Niedobitek and Young, 1997).

The incidence and clinical presentation of PTLDs varies with the organ transplanted, the duration of immunosuppression and the dosage and number of agents used, although there are a number of common clinical features, which include their frequent occurrence in multiple extranodal locations such as the gastrointestinal tract or even in the allograft organ itself. The high incidence of PTLDs in the transplanted organ suggests that chronic antigen stimulation in the graft might be important in the pathogenesis of these lesions. Indeed, T cells are required for the development of PTLD-like tumours in severe combined immunodeficient (SCID) mice, suggesting an important role for T cell help in the growth of B cell derived PTLDs (Johannsen *et al.,* 2000).

The majority of PTLD cases are EBV-positive and many show a Lat III pattern of gene expression (Young *et al.,* 1989). Thus, in many cases, PTLDs appear to represent the in vivo counterpart of *in vitro* immortalised LCLs and, by implication, are likely to be primarily driven by EBV. However, other forms of EBV latency, in which EBNA2 or LMP1 are not expressed, are frequently observed particularly in monoclonal tumours suggesting that these lymphomas have evolved from EBV-transformed LCL-like lesions which have acquired additional cellular genetic changes that render certain viral functions redundant.

Hodgkin's lymphoma

Although epidemiological evidence suggested that HL might be caused by an infectious agent (MacMahon, 1966) and serological studies implicated EBV infection (Levine *et al.,* 1971; Mueller *et al.,* 1989), it was the direct detection of EBV genomes, transcripts and proteins in the malignant Hodgkin/Reed-Sternberg (HRS) cells that provided the most convincing evidence (Wu *et al.,* 1990; Pallesen *et al.,* 1991; Weiss et al. 1991). Thus, around 40% of classical HL in the West is EBV-associated with the mixed cellularity and lymphocyte-depleted histological types being most commonly infected. The proportion of EBV-positive HL varies with age, ethnicity and geographical location. HL in older patients (>55 years of age) and in children, especially boys under 10 years, has been shown to be more likely to be EBV-associated than HD in young adults (Armstrong *et al.,* 1998). EBV-positive HL also affects more Asians and Hispanics than whites or blacks (Glaser *et al.,* 1997) and there is a strong association between EBV-positivity and South Asian ethnicity in paediatric HD patients (Flavell *et al.,* 2001). Whilst an unusual response to primary EBV infection might account for the incidence of virus-positive HL cases in the young age group, the association of EBV with the tumour in older patients could reflect increased EBV activity as a result of failing T cell immunity. In this respect the overall incidence of HL is marginally increased in AIDS patients, but the majority of HL tumours arising in AIDS patients are EBV-associated (Uccini *et al.,* 1990).

The monoclonal EBV genome is present in every HRS cell within the tumour and expresses a particular subset of latent cycle proteins, EBNA1, LMP1 and LMP2, the so-called Latency II programme (Deacon *et al.,* 1993). The identification by immunoglobulin (Ig) genotyping of HRS cells as failed products of germinal centre reactions (Kanzler *et al.,* 1996; Kuppers, 2002) suggests that

EBV infection may be rescuing such tumour progenitors from apoptosis. This scenario is supported by the ability of LMP1 to constitutively activate the CD40 pathway, thereby replacing the signal that is normally provided by cognate T cells during memory B cell selection, and by LMP2A mimicking the signal from surface Ig, replacing the usual requirement for high affinity binding to cognate antigen (Young& Rickinson, 2004). Constitutive activation of the NF-κB pathway appears to be a vital step in HL pathogenesis which can be achieved either by LMP1 in EBV-positive tumours or by genetic modification via inactivation of IκBα or amplification of the REL gene (Kuppers, 2002).

Burkitt's lymphoma

The recognition that Burkitt's lymphoma (BL) in Africa was apparently restricted to areas where infection with *Plasmodium falciparum* malaria was holoendemic led to the suggestion that an infectious agent might be involved and eventually to the discovery of EBV. The so-called 'endemic' or high-incidence form of BL occurs at an annual incidence of approximately 5-10 cases per 100, 000 children in equatorial Africa and parts of Papua New Guinea. By contrast, sporadic cases of BL occur worldwide but at a much lower frequency (at least 50-fold less than in the high-incidence areas). Whereas virtually every BL tumour found in the high-incidence regions of Africa is EBV positive, only about 15% of sporadic BL tumours carry the virus (Rickinson and Kieff, 2001). In addition, certain 'intermediate–incidence' areas outside the regions of holoendemic malaria, such as Algeria and Egypt have increased numbers of cases that correlate with an increased proportion of EBV-positive tumours. BL is also observed as a consequence of HIV infection, frequently occurring before the development of full-blown AIDS. Only 30-40% of cases of AIDS-BL are associated with EBV infection. A consistent feature of all BL tumours, irrespective of geographical location or AIDS association, are chromosomal translocations involving the long arm of chromosome 8 (8q24) in the region of the c-*myc* proto-oncogene and either chromosome 14 in the region of the immunoglobulin heavy-chain gene or, less frequently, chromosomes 2 or 22 in the region of the immunoglobulin light-chain genes. This translocation results in deregulated expression of the c-*myc* oncogene.

The precise role of EBV in the pathogenesis of BL remains to be established, although the detection of monoclonal EBV episomes in virus-positive BL biopsies suggests that EBV infection preceded proliferation of the precursor B cells (Neri *et al.,* 1991). The majority of EBV-positive tumours show a highly restricted Latency I form of infection with viral antigen expression limited to EBNA1 (Rowe *et al.,* 1987; Kelly *et al.,* 2002). This coupled with the germinal centre B cell phenotype of BL and the presence of deregulated myc expression suggests a complex scenario in which EBV-transformed B cells may provide a pool of target cells at increased risk of undergoing the c-myc translocation and/or a direct role for Latency I-active EBV genes in the oncogenic process. In vitro studies have highlighted the apparent incompatibility of the EBV Latency III-driven and of the c-myc-driven growth programmes in B cells (Polack *et al.,* 1996; Pajic *et al.,* 2001), suggesting that the evolution to BL can only occur if the EBV programme is suppressed. This selection against full EBV latent gene expression is further supported by a subset of BLs that retain transcriptional features of Latency III but where the resident viral genome has deleted the EBNA2 gene and therefore abrogated conventional B cell transforming function (Kelly *et al.,* 2002).

T cell and NK cell lymphomas

Under certain rare circumstances EBV is able to infect T cells and NK cells resulting in lymphomas. These tumours are most common in Asian populations and appear to arise either after acute primary infection, manifesting as virus-associated haemophagocytic syndrome (VAHS), or as a consequence of chronic active EBV infection with VAHS-like symptoms (Jones *et al.,* 1988; Kanegane *et al.,* 2002). A proportion of these malignancies are monoclonal EBV genome-positive T cell lymphomas which originate from either CD4+ or CD8+ T cells and express EBNA1 and LMP2 with variable levels of LMP1 (Niedobitek and Young, 1997; Rickinson and Kieff, 2001). Others are extranodal lymphomas of T cell (CD3+, CD56+/-) or NK cell origin (CD3-, CD56+) which present as aggressive nasal ('lethal midline granuloma') tumours (Harabuchi *et al.,* 1990; Kanavaros *et al.,* 1993).

EBV-associated carcinomas

EBV infection is also associated with the development of nasopharyngeal carcinoma (NPC), a subset of gastric adenocarcinomas and of certain salivary gland carcinomas (Rickinson and Kieff, 2001; Raab-Traub, 2002). The EBV-associated undifferentiated form of nasopharyngeal carcinoma (WHO type III) shows the most consistent worldwide association with EBV and is common in areas of China and South-East Asia, being particularly prevalent in Cantonese males. This tumour appears to arise as a consequence of an ethnic predisposition, EBV infection and environmental co-factors such as dietary components (e.g. salted fish) (Yu *et al.,* 1986). NPC tumours are characterised by the presence of undifferentiated carcinoma cells together with a prominent lymphocytic infiltrate and this interaction between tumour cells and lymphocytes appears to be crucial for the continued propagation of the malignant component. EBV latent gene expression in NPC is predomi-

nantly restricted to the EBNA1 nuclear antigen, the latent membrane proteins (LMP2A, LMP2B) and the *Bam*HIA transcripts with around 20% of tumours also expressing the oncogenic LMP1 protein (Raab-Traub, 2002). Studies of normal nasopharynx and premalignant biopsies indicate that genetic events occur early in the pathogenesis of NPC and that these may predispose to subsequent EBV infection (Young and Rickinson, 2004). EBV is also found in around 10% of more typical gastric adenocarcinomas accounting for up to 75,000 new cases per year (Shibata and Weiss, 1992; Tokunaga *et al.*, 1993). These tumours display a restricted pattern of EBV latent gene expression (EBERs, EBNA1, LMP2A, BARTs, BARF1) similar to that observed in NPC (Imai *et al.*, 1994). There is significant geographical variation in the association of EBV with gastric carcinoma which may be attributed to ethnic and genetic differences. EBV-positive gastric carcinomas have distinct phenotypic and clinical characteristics compared to EBV-negative tumours including loss of p16 expression and improved patient survival (Schneider *et al.*, 2000; Lee *et al.*, 2004). As in NPC, the precise role of EBV in the pathogenesis of gastric carcinoma remains to be determined but the absence of EBV infection in premalignant gastric lesions supports the contention that virus infection is a relatively late event in gastric carcinogenesis (zur Hausen *et al.*, 2004).

Conclusions

Substantial evidence implicates EBV in the pathogenesis of tumours arising in both lymphoid and epithelial tissues. The virus adopts different forms of latent infection in different tumour types, reflecting the complex interplay between EBV and the host cell environment. Studies of the function of individual EBV latent genes have highlighted the ability of these proteins to target specific cell signalling pathways. This knowledge is not only providing insights into the fundamental mechanisms regulating cell growth and survival but is also suggesting novel approaches to the treatment of EBV-associated tumours. EBV was discovered in 1964 and its genome was fully sequenced in 1984. Having had access to this DNA sequence for over 20 years, we are just beginning to appreciate the complexity of this relatively small (172 kb) piece of DNA. These lessons are important in considering the impact of the human genome sequence (some $3x10^6$ kb) on our understanding of cell biology and disease.

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