

# ONCOGENIC $\gamma$ -HERPESVIRUSES: COMPARISON OF VIRAL PROTEINS INVOLVED IN TUMORIGENESIS

*Blossom Damania*

Herpesviruses are present in most species throughout the animal kingdom and are classified into three subfamilies,  $\alpha$ ,  $\beta$  and  $\gamma$ , on the basis of their biological properties and genome sequences. A striking feature that is shared by many of the  $\gamma$ -herpesviruses is their ability to induce neoplastic disease in the host. This review focuses on three  $\gamma$ -herpesviruses: Epstein–Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV) and herpesvirus saimiri (HVS), and discusses the diverse array of EBV, KSHV and HVS viral genes that are involved in transformation, cell signalling, episomal maintenance and cell proliferation.

## NEOPLASIA

Abnormal new growth of cells in the process of tumour formation.

## PARACRINE

A substance that is secreted by a cell and which acts on neighbouring cells

## LYMPHOMA

A general term for cancers that develop in the lymphatic system.

## LYMPHOSARCOMA

A malignant lymphoma.

## LEUKAEMIA

Cancer of white blood cells.

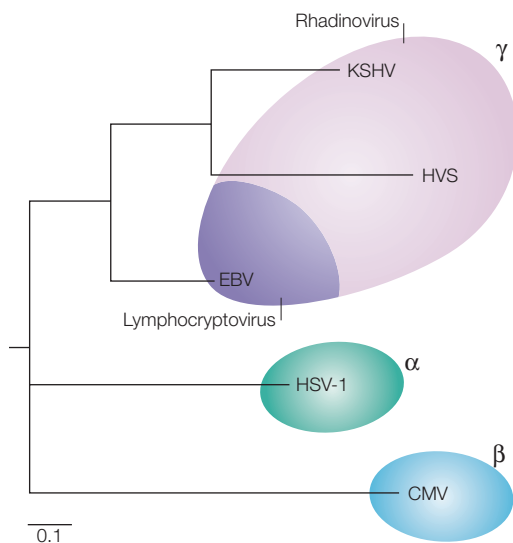
*Lineberger Comprehensive Cancer Center, Department of Microbiology & Immunology, CB #7295, University of North Carolina, Chapel Hill, North Carolina 27599, USA.*  
e-mail: [damania@med.unc.edu](mailto:damania@med.unc.edu)  
doi:10.1038/nrmicro958

Herpesviruses are double-stranded DNA viruses that are prevalent throughout the animal kingdom. Most animal species can be infected by at least one herpesvirus, and more than 130 herpesviruses have been identified so far. In humans, eight herpesviruses have been identified: herpes simplex virus 1 and 2 (**HSV-1** and **HSV-2** or human herpesvirus (HHV)-1 and HHV-2); varicella-zoster virus (**VZV** or HHV-3); Epstein–Barr virus (**EBV** or HHV-4); human cytomegalovirus (**HCMV** or HHV-5); human herpesviruses 6 and 7 (**HHV-6** and **HHV-7**); and Kaposi's sarcoma-associated herpesvirus (**KSHV** or HHV-8). All herpesviruses share a common evolutionary origin, as evidenced by the high amino acid sequence similarity that is observed among many of their viral gene products.

The herpesviruses are classified into three subfamilies,  $\alpha$ ,  $\beta$  and  $\gamma$ , on the basis of their genome sequence and organization, and biological characteristics. The  $\gamma$ -subfamily of herpesviruses is lymphotropic and some are capable of undergoing lytic replication in epithelial and/or fibroblast cells. These viruses establish a lifelong period of latency in their host, with intermittent periods of lytic replication. The  $\gamma$ -herpesvirinae have been identified in many different animal species<sup>1</sup>. There are two subfamilies of  $\gamma$ -herpesviruses: the lymphocryptoviruses ( $\gamma$ -1) and the rhadinoviruses ( $\gamma$ -2). EBV<sup>2</sup> is a lymphocryptovirus, whereas herpesvirus saimiri

(HVS)<sup>3</sup>, which infects monkeys, and KSHV<sup>4</sup> are rhadinoviruses. The  $\gamma$ -herpesviruses share more genes with each other than with members of either the  $\alpha$ - or  $\beta$ -subfamilies of herpesviruses (FIG. 1), and their genomes are organized in a relatively similar fashion (FIG. 2).

A striking property that is shared by many members of the  $\gamma$ -herpesvirus family, including EBV, KSHV and HVS, is their ability to induce NEOPLASIA in natural or experimental hosts (TABLE 1). All three viruses are associated with different types of malignancies in either their natural host (EBV and KSHV) or a foreign host (HVS). All three viruses have also been associated with lymphoproliferative diseases of B and/or T cells. Additionally, EBV is associated with epithelial cancers such as nasopharyngeal carcinoma (NPC) and KSHV is linked to vascular endotheliosarcomas such as Kaposi's sarcoma (KS) (TABLE 1). Current evidence indicates that both the latent and lytic genes of these herpesviruses contribute to viral oncogenesis through a transforming and/or PARACRINE mechanism, causing normal cells to proliferate in an uncontrolled manner. HVS is non-pathogenic in its natural host, the squirrel monkey, and can establish a lifelong period of persistence, primarily in T lymphocytes<sup>3</sup>. Cross-species transmission of HVS into another New World primate, such as the common marmoset, fosters the development of LYMPHOMAS, LYMPHOSARCOMAS and LEUKAEMIAS<sup>5</sup>. Similar to



**Figure 1 | A phylogenetic tree representing the  $\gamma$ -herpesviruses Epstein-Barr virus (EBV), Kaposi's sarcoma-associated virus (KSHV) and herpesvirus saimiri (HVS).** The tree was constructed using full-length DNA polymerase gene sequences of the indicated herpesviruses. KSHV and HVS (rhadinoviruses) and EBV (lymphocryptovirus) are members of the  $\gamma$ -herpesvirus subfamily, whereas herpes simplex virus 1 (HSV-1) is an  $\alpha$ -herpesvirus and cytomegalovirus (CMV) is a  $\beta$ -herpesvirus. Sequences were aligned using the ClustalW program, and the Phylodendron program was used to construct the phenogram. NCBI GenBank accession numbers for DNA polymerase genes used in the construction of the tree were: KSHV (U75698), EBV-B95.8 (V01555), HVS (M31122), HSV-1 (X14112) and CMV (BK000394).

the human viruses EBV and KSHV, the primate  $\gamma$ -herpesvirus HVS is also lymphotropic and capable of inducing T-cell neoplasia.

In common with other herpesviruses, the  $\gamma$ -herpesviruses remain latent in their natural host, where the viral genome exists as a closed circular molecule, and only a fraction of the viral genes are expressed. A subset of these latent viruses can reactivate and enter the lytic cycle. During the lytic phase of the herpesvirus life cycle there is a temporal order of viral gene expression, and the viral genome is replicated many times, which leads to the production of infectious virion progeny and the death of the infected cell. Although differentiating the expression profiles of viral genes into lytic versus latent genes is useful for classification purposes, many  $\gamma$ -herpesvirus genes are transcribed in both phases of the viral life cycle — for example, EBV latent membrane protein 1 (LMP1) and KSHV Kaposin are expressed during latency as well as during the lytic cycle of EBV and KSHV, respectively. In addition, depending on the type of viral malignancy, most infected cells in the tumour can express one subset of genes, whereas a fraction of the tumour cells express a different set of viral genes. For example, most cells in a KS lesion express the latency-associated nuclear antigen (LANA) but a small percentage of cells express the viral G-protein coupled receptor (vGPCR), which is encoded by a lytic gene

(TABLE 2). So, the complex nature of the biological properties and associated malignancies of the  $\gamma$ -herpesvirinae requires study of viral gene expression in the context of both the cell type and the cell environment. This intricate complexity is not surprising as these viruses encode a plethora of proteins that enable the virus to survive successfully in the host environment. In the case of EBV, the expression patterns of viral latent genes in EBV-associated malignancies have been well documented, and these characteristic profiles are classified as Type I, II or III latency (BOX 1). Although viral genes that are transcribed during latency are generally considered to be essential for the transformation process and for the development of viral-induced neoplasia, it has recently become apparent that a subset of lytic genes can also contribute to the development of viral-induced cancers through a paracrine mechanism.

Given the oncogenic disease manifestations shown by these  $\gamma$ -herpesviruses in their natural and experimental hosts, it seems likely that they use common strategies to induce cell proliferation, transformation and tumorigenesis, and to evade the antiviral host response. This review describes several important transforming and signalling proteins of EBV, KSHV and HVS that might contribute to cellular transformation. Viral cytokines that can augment the neoplastic process are also discussed.

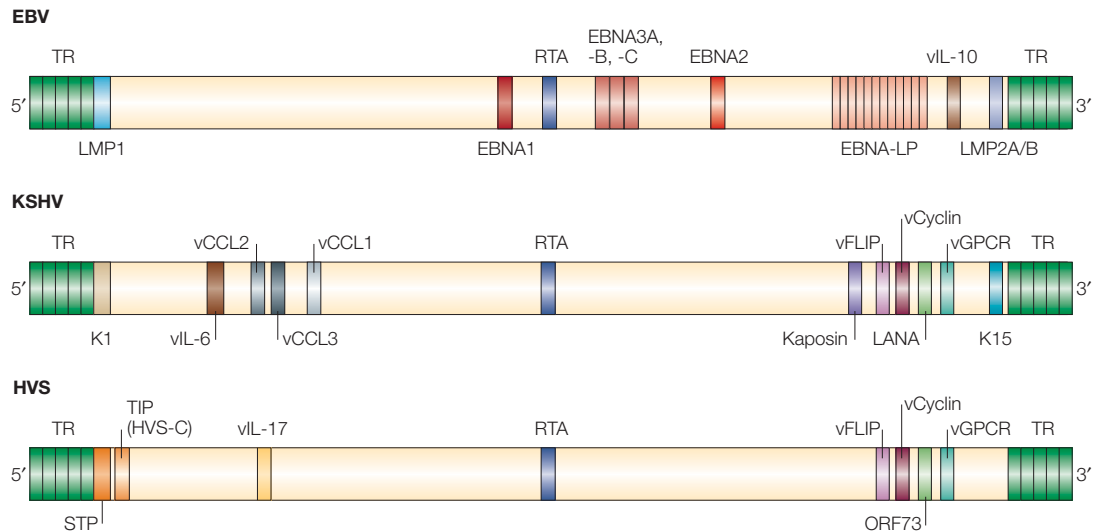
#### Viral transforming and signalling proteins

The  $\gamma$ -herpesviruses encode a plethora of viral transforming and signalling proteins that are capable of modulating host cell signalling pathways to protect and preserve the virus-infected cell. These viral modulators include unique viral proteins that have no discernable amino acid sequence similarity to cellular proteins, as well as homologues of cellular proteins that have sequence similarity to their cellular counterparts. Many of these viral transforming and signalling proteins are described below in further detail.

A remarkable property shared by the three  $\gamma$ -herpesviruses EBV, KSHV and HVS is that they all contain a unique open reading frame (ORF) at the 5' end of their respective genomes, each of which possesses transforming potential (FIG. 3, TABLE 3). Although these genes have highly divergent sequences, the functions of the gene products seem to be conserved and they can activate B- and/or T-cell signalling pathways, leading to cellular proliferation (FIG. 3).

**EBV LMP1.** The first EBV ORF encodes the viral oncoprotein LMP1. EBV LMP1 can transform rodent fibroblasts, which, when injected into nude mice, can generate multiple tumours<sup>6</sup>. LMP1 has been shown to inhibit terminal differentiation in epithelial cells and is also essential for the immortalization of primary human B lymphocytes in which LMP1 is expressed to LYMPHBLASTOID CELL LINES (LCLs, which exhibit a Type III latency profile; BOX 1)<sup>7,8</sup>. Furthermore, transgenic mice expressing LMP1 under the control of the immunoglobulin promoter have an increased frequency of B-cell lymphomas<sup>9</sup>. Expression of LMP1 induces several

LYMPHBLASTOID CELL LINES  
Virally infected lymphocytes propagated in tissue culture become enlarged, appear activated, and continuously divide.



**Figure 2 | Alignment of the genomes of three  $\gamma$ -herpesviruses: Epstein–Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV) and herpesvirus saimiri (HVS).** The alignment is shown with respect to the genes discussed in this review. The first (LMP1, K1 and STP) and last (LMP2A/B and K15) open-reading frames abutting the terminal repeats are indicated. Other viral genes involved in transformation and modulation of signalling and maintenance of latency are also indicated, together with the viral cytokines. EBNA, EBV nuclear antigen; GPCR, G-protein-coupled receptor; IL, interleukin; LANA, latency-associated nuclear antigen; LMP, latent membrane protein; STP, saimiri transformation protein; TIP, tyrosine-kinase-interacting protein; TR, terminal repeats.

pleiotropic effects, including the upregulation of adhesion molecules, anti-apoptotic proteins and cytokines.

EBV LMP1 has six transmembrane-spanning domains, a short amino terminus and a 199-amino-acid cytoplasmic tail. Functionally, LMP1 mimics the CD40 receptor, which is a marker of B-lymphocyte activation and is involved in the regulation of antibody production, isotype switching and clonal expansion of B cells<sup>10–12</sup>. However, unlike CD40, signalling by LMP1 is constitutive so the transduction of signals occurs in the absence of extracellular ligands. The carboxyl terminus of LMP1 can be divided into two regions — C-terminal activation regions 1 and 2 (CTAR1 and CTAR2) — both of which are necessary for nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation<sup>13–15</sup> (FIG. 3). NF- $\kappa$ B belongs to a family of transcription factors that regulate a wide range of cellular pathways, including cell proliferation, immunity and apoptosis. The LMP1 C-terminal domain can interact with tumour-necrosis factor (TNF)-receptor-associated

factors (TRAFs), with TNF-receptor-associated death domains (TRADDs) and with receptor-interacting protein (RIP)<sup>16–20</sup> (FIG. 3). The interactions of LMP1 with TRAFs and TRADDs are essential for the activation of the NF- $\kappa$ B pathway and for EBV-induced immortalization of B lymphocytes, which indicates that activation of the NF- $\kappa$ B pathway by LMP1 might be required for sustained proliferation of EBV-infected cells<sup>15,21</sup>. Additionally, LMP1 has also been shown to activate the phosphatidylinositol 3-kinase (PI3K) and Jun kinase (JNK) pathways<sup>22,23</sup>. Although activation of PI3K by LMP1 can help suppress apoptosis and promote cell survival, the activation of the JNK pathway can induce expression of proteins that are involved in cell proliferation and transformation. The interaction of LMP1 with TRAF3 results in the localization of LMP1 with lipid rafts, and the transmembrane domain of LMP1 has also recently been shown to be crucial for raft localization and LMP1 signalling<sup>24,25</sup>. Interestingly, despite constitutive association with TRADDs and RIP, LMP1 does not induce apoptosis in epithelial or B cells<sup>18</sup>. So, it seems that LMP1 contributes to EBV-induced transformation of primary B lymphocytes by mimicking the activation function of the CD40 receptor.

In epithelial cells, LMP1 has been shown to induce the expression of the pro-angiogenic factors vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) together with cyclooxygenase-2 (COX-2) and matrix metalloproteinase 9 (MMP-9)<sup>26–28</sup>. All these proteins are thought to have a role in angiogenesis, a process that involves the formation of new blood vessels. Many tumour cells express high concentrations of these angiogenic factors to attract blood vessels that can feed the tumour itself. Hence, by inducing

**Table 1 | Primate  $\gamma$ -herpesviruses linked to neoplastic disease**

$\gamma$ -herpesvirus	Natural host	Associated malignancies
Epstein–Barr virus	Human	Burkitt's lymphoma Hodgkins' lymphoma Post-transplant lymphoma X-linked lymphoproliferative syndrome T-cell lymphomas Nasopharyngeal carcinoma Gastric carcinoma
Kaposi's sarcoma-associated herpesvirus	Human	Kaposi's sarcoma Primary effusion lymphomas Multicentric Castlemans disease
Herpesvirus saimiri	Squirrel monkey	T-cell leukaemia in non-natural host T-cell lymphomas in non-natural host

Table 2 | A subset of viral proteins expressed in KSHV-associated tumour samples

Malignancy	Proteins expressed in most tumour biopsies	Proteins expressed in few tumour biopsies
Kaposi's sarcoma	LANA, vCyclin, vFLIP, vIRF-1, Kaposin	vIL-6, K1, vGPCR
Primary effusion lymphomas	LANA, vCyclin, vFLIP, vIRF-3/LANA-2, Kaposin, vIL-6 (~40%)	K1, vGPCR
Multicentric Castlemans disease	LANA, vCyclin, vFLIP, vIL-6 (~40%)	K1, vGPCR

FLIP, FLICE inhibitory protein; GPCR, G-protein-coupled receptor; IL, interleukin; IRF, interferon regulatory factor; KSHV, Kaposi's sarcoma-associated herpesvirus; LANA, latency-associated nuclear antigen.

these secreted angiogenic factors, LMP1 functions in a paracrine manner in epithelial cells to augment transformation. As in B cells, LMP1 also stimulates the NF- $\kappa$ B pathway in epithelial cells and in doing so might contribute to the development of NPC<sup>14,29,30</sup>.

**KSHV K1.** The first ORF of the KSHV genome encodes a gene product known as K1. Like EBV LMP1, KSHV K1 can transform rodent fibroblasts, and these transformed cells can form tumours in nude mice<sup>31</sup>. K1 can also functionally substitute for HVS saimiri transformation protein (STP) in the immortalization of common marmoset T lymphocytes to interleukin (IL)-2-independent growth<sup>31</sup> and in the induction of lymphomas in these animals<sup>31</sup>. As normal primary T cells require IL-2 to grow, this indicates that both STP and K1 can activate pathways that promote primary T-cell growth by a mechanism that is not dependent on the addition of exogenous cytokines. A small percentage of transgenic mice expressing K1 under the control of the simian virus 40 promoter developed spindle-cell sarcomas and malignant PLASMABLASTIC LYMPHOMAS after 14 months<sup>32</sup>. B lymphocytes from these K1-transgenic mice showed constitutive activation of the NF- $\kappa$ B and octamer-binding factor 2 (Oct-2) transcription factors, and showed increased activity and phosphorylation of the tyrosine kinase LYN, indicating that K1-induced superactivation of these cellular transcription factors and kinases directly contributes to the development of B-cell lymphomas<sup>32</sup>.

K1 is a transmembrane glycoprotein that contains an N-terminal extracellular domain, a transmembrane region and a short cytoplasmic tail at the C-terminus<sup>31,33</sup> (FIG. 3). The cytoplasmic domain contains a functional immunoreceptor tyrosine-based activation motif (ITAM)<sup>34,35</sup>, which can transduce signals to induce calcium mobilization, tyrosine phosphorylation of cellular kinases and activation of NF- $\kappa$ B and nuclear factor of activated T cells (NFAT), all of which are indicative of lymphocyte activation. However, unlike other

ITAM-based signal-transduction events, which require a ligand-receptor interaction, K1 signalling, similar to EBV LMP1 signalling, is constitutive<sup>31,34,35</sup>.

In B lymphocytes, the K1 protein induces phosphorylation of several cellular signal-transduction proteins, including VAV, p85, SYK and AKT kinase<sup>34-36</sup>. In addition to activating B cells, K1 can also protect these cells from Fas-induced apoptosis by preventing the induction of Fas ligand expression<sup>36</sup>. K1 is expressed in KS lesions<sup>37,38</sup>, as well as in MULTICENTRIC CASTLEMAN'S DISEASE (MCD) and PRIMARY EFFUSION LYMPHOMAS (PELs)<sup>39</sup>. K1 mRNA has been detected in whole-tumour samples of KS lesions<sup>38</sup> and expression of K1 in endothelial and epithelial cells results in the upregulation and secretion of VEGF and MMP-9, which is similar to the situation seen with EBV LMP1 (REF. 40). Thus, K1 seems functionally similar to EBV LMP1 in B lymphocytes, where it can activate B-cell signalling pathways, whereas in endothelial cells, K1 might contribute to KSHV pathogenesis through a paracrine mechanism that promotes tumour progression and growth.

**HVS STP.** There are three different HVS subgroups (A, B and C), which differ in oncogenic potential. Subgroups A and C can immortalize common marmoset T lymphocytes to IL-2-independent growth, whereas subgroup B is non-oncogenic. In all three subgroups, the first ORF of the HVS genome codes for the STP protein<sup>41</sup> and the transforming ability of STP directly correlates with the oncogenic potential of the different viral subgroups. Deletion of the STP gene from the genomes of subgroups A and C renders these viruses capable of replication but unable to induce lymphomas in common marmosets or transform T lymphocytes *in vitro*<sup>42,43</sup>. Similar to LMP1 and K1, STP from HVS subgroup C (STP-C) can transform Rat-1 cells, resulting in foci formation and induction of invasive tumours in nude mice<sup>43,44</sup>. In addition, transgenic mice expressing STP-C developed extensive epithelial cell tumours and lymphomas<sup>43,44</sup>.

Both the STP-A and STP-C proteins comprise an acidic N-terminus, collagen-like repeats in the central region and a hydrophobic C-terminus (FIG 3). The collagen-like repeats seem to be important for the induction of NF- $\kappa$ B activity and for the transformation potential of STP-C. Indeed, when substituted into the non-transforming *stp* gene from subgroup B, these repeats can render it transforming<sup>45</sup>. STP-C can associate with cellular RAS<sup>46</sup> and this interaction is crucial for its transforming activity in cell culture. Analogous to EBV LMP1, STP-C has also been shown to activate NF- $\kappa$ B transcriptional activity by interacting with TRAF1, -2, and -3 (REF. 47) (FIG. 3).

#### PLASMABLASTIC LYMPHOMAS

A form of malignant B-cell lymphoma in which the cells resemble antibody-secreting plasma cells.

#### MULTICENTRIC CASTLEMAN'S DISEASE

A rare B-cell lymphoproliferative disorder.

#### PRIMARY EFFUSION LYMPHOMAS

A subset of malignant B-cell lymphomas that localize to body cavities such as the pleura, peritoneum or pericardium.

### Box 1 | Viral latent genes expressed in EBV-associated malignancies

#### Burkitt's lymphoma (Type I latency)

EBNA1 | EBERs | BARTs

#### Hodgkin's lymphoma (Type II latency)

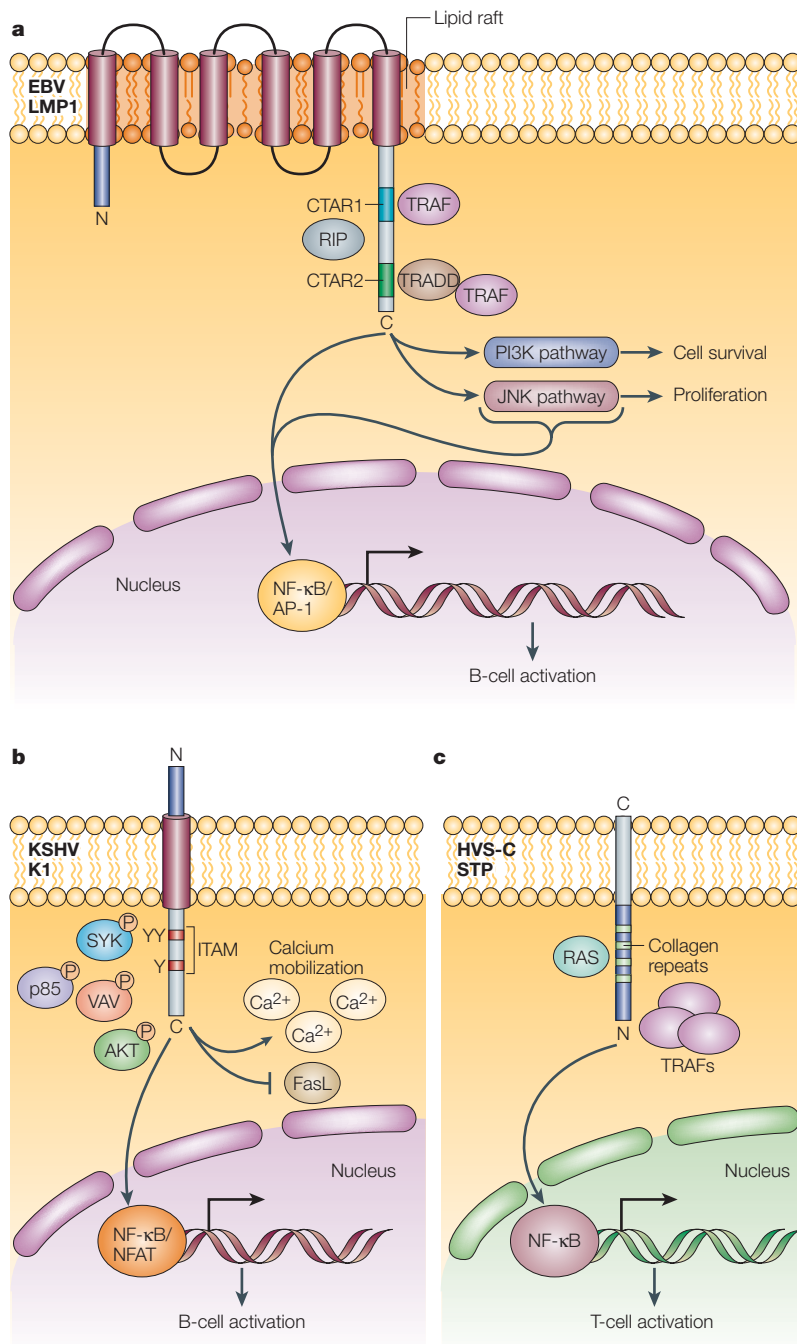
EBNA1 | LMP1, -2 | EBERs | BARTs

#### Post-transplant lymphoma (Type III latency)

EBNA1, -2, -3A, -B, -C, -LP | LMP1, -2 | EBERs | BARTs

#### Nasopharyngeal carcinoma (Type II latency)

EBNA1 | LMP1, -2 | EBERs | BARTs



**Figure 3 | Transforming proteins encoded by the first open reading frames of the  $\gamma$ -herpesviruses EBV, KSHV and HVS.** These proteins — EBV LMP1, KSHV K1 and HVS STP — are involved in activating B- or T-cell signalling pathways and contain domains that can interact with cellular factors as indicated. **a** | EBV LMP1 can interact with multiple TRAFs and TRADDs leading to B-cell activation through the induction of the cellular transcription factors AP-1 and NF- $\kappa$ B. The PI3K and JNK pathways are also activated. **b** | KSHV K1 can mobilize calcium and activate SYK, VAV, p85 and AKT kinase leading to B-cell activation through the induction of the cellular transcription factors NFAT and NF- $\kappa$ B. K1 can also protect cells from Fas-induced apoptosis by preventing the induction of expression of the Fas ligand. **c** | HVS-C STP can activate RAS and associate with TRAFs to activate T cells through the induction of the cellular transcription factor NF- $\kappa$ B. EBV, Epstein–Barr virus; HVS, herpesvirus saimiri; JNK, Jun N-terminal kinase; KSHV, Kaposi's sarcoma-associated herpesvirus; LMP, latent membrane protein; NFAT, nuclear factor of activated T cells; NF- $\kappa$ B, nuclear factor  $\kappa$ B; PI3K, phosphatidylinositol 3-kinase; STP, saimiri transformation protein; TRADD, tumour-necrosis-factor-receptor-associated death domain; TRAF, tumour-necrosis-factor-receptor-associated factor.

**Signal modulators of the B- and T-cell receptors**

Antibody cross-linking of the B-cell antigen receptor (BCR), and major histocompatibility complex (MHC)-mediated stimulation of the T-cell antigen receptor (TCR), initiates a signal-transduction cascade that results in lymphocyte activation. It is currently believed that cellular activation of virus-infected lymphocytes often triggers viral reactivation. EBV LMP2A, KSHV K15 and HVS tyrosine-kinase-interacting protein (TIP) have all been shown to dampen or abrogate the BCR and TCR signalling pathways and, by doing so, might help to prevent viral reactivation from latently infected lymphocytes at an inopportune moment. The LMP2A, K15 and TIP proteins are all capable of associating with the major B- or T-cell-receptor-associated kinases and antagonizing their signalling activities (FIG. 4, TABLE 3). It is postulated that these proteins help to maintain viral latency in the host.

**EBV LMP2A.** The gene that encodes LMP2A is located at the 3' end of the linear EBV genome (FIG. 2). LMP2A is a 12-transmembrane-spanning-domain protein with short N- and C-terminal tails (FIG. 4) and is expressed in EBV-infected epithelial and B cells, where it aggregates in the plasma membrane<sup>48,49</sup>. Like KSHV K1, the N-terminal cytoplasmic region of LMP2A contains several tyrosine-based SRC homology 2 (SH2)-domain-binding sites, two of which form a functional ITAM<sup>50</sup>. However unlike K1, LMP2A inhibits normal BCR signalling<sup>51</sup> in EBV-negative B cells by preventing the BCR from entering lipid rafts and interacting with LYN kinase<sup>50–53</sup>. In addition, studies using EBV-positive B lymphocytes have shown that this LMP2A-induced signalling block prevents the reactivation of lytic replication, indicating that EBV LMP2A might have an important role in the establishment and maintenance of viral latency *in vivo*<sup>53</sup>. LMP2A has also been shown to block apoptotic signals in B lymphocytes through its activation of the PI3K/AKT pathway, resulting in the promotion of a cell-survival signal<sup>54</sup>. Transgenic mice that express LMP2A have retarded B-cell development, causing immunoglobulin-negative B cells to locate to peripheral lymphoid organs<sup>55</sup>. This indicates that LMP2A probably mediates survival of immature EBV-infected B cells in the absence of functional BCR signalling, thereby bypassing host-cell control.

Although LMP2A is dispensable for EBV-immortalization of B lymphocytes<sup>56</sup>, it induces a hyperproliferative response and prevents differentiation in epithelial cells<sup>57</sup>. LMP2A-expressing epithelial cells can form colonies in soft agar and induce aggressive tumours in nude mice<sup>57</sup>. In keratinocytes, LMP2A activates both the PI3K and  $\beta$ -catenin signalling pathways<sup>58</sup>. As these pathways have been linked to cell survival and cell proliferation, respectively, LMP2A probably contributes to EBV tumorigenicity in epithelial cells by promoting transformation and preventing apoptosis of the infected epithelial cell<sup>58</sup>.

Table 3 | Common and unique functions of proteins encoded by EBV, KSHV and HVS

Viral protein			Function
EBV	KSHV	HVS (A11/C488)	
LMP1	K1	STP (of A and C subgroups)	Transforming and signalling protein
LMP2A	K15	TIP (only in subgroup C)	Signal modulator
EBNA1	LANA	ORF73	Episomal maintenance
EBNA2	–	–	Nuclear protein, transcription factor
EBNA3A	–	–	Nuclear protein, transcription factor
EBNA3B	–	–	Nuclear protein, transcription factor
EBNA3C	–	–	Nuclear protein, transcription factor
–	Kaposin	–	Transforming protein
BCRF1	–	–	IL-10 homologue
–	K2	–	IL-6 homologue
–	–	ORF13	IL-17 homologue
–	ORF74	ORF74	GPCR homologue
–	ORF72	ORF72	Cyclin homologue

BCRF, *Bam*H1 C fragment rightward reading frame 1; EBNA, EBV nuclear antigen; EBV, Epstein–Barr virus; GPCR, G-protein-coupled receptor; HVS, herpesvirus saimiri; IL, interleukin; KSHV, Kaposi's sarcoma-associated herpesvirus; LANA, latency-associated nuclear antigen; LMP, latent membrane protein; TIP, tyrosine-kinase interacting protein.

**KSHV K15.** Similar to the situation in EBV, the gene encoding the KSHV K15 viral signal modulatory protein is positioned at the 3' end of the KSHV genome, and is located in an isologous genomic position to the EBV LMP2A gene<sup>59,60</sup> (FIG. 2). K15 isolates have a complex splicing pattern with all isoforms containing 4–12 transmembrane-spanning domains and a short cytoplasmic tail. The functions of the various K15 isoforms are currently under investigation<sup>59–61</sup>. K15 is weakly expressed in latently infected PELs and the level of its expression is markedly increased on lytic reactivation with phorbol esters<sup>59,60</sup>. The K15 cytoplasmic tail contains SH2- and SH3-binding motifs and a YASIL (Tyr–Ala–Ser–Iso–Leu) sequence that seem to be necessary for activation of the NF- $\kappa$ B and mitogen-activated protein kinase (MAPK) pathways; however, the effects of activation of these pathways by K15 are not yet fully understood<sup>59,60,62</sup>. The K15 cytoplasmic tail is constitutively tyrosine phosphorylated and can interact with cellular tyrosine kinases and TRAFs<sup>59,60,62</sup> (FIG. 4). In common with EBV LMP2A, the cytoplasmic domain of KSHV K15 can inhibit BCR signal transduction<sup>60</sup> (FIG. 4). The ability of K15 to antagonize BCR signalling events might preserve viral latency, as activation of the B cell through BCR signalling is one possible mechanism for viral reactivation. Finally, similar to EBV LMP1 and LMP2A, the K15 protein can localize to lipid rafts<sup>62</sup> and might use a mechanism that is similar to EBV LMP2A to inhibit BCR signal transduction.

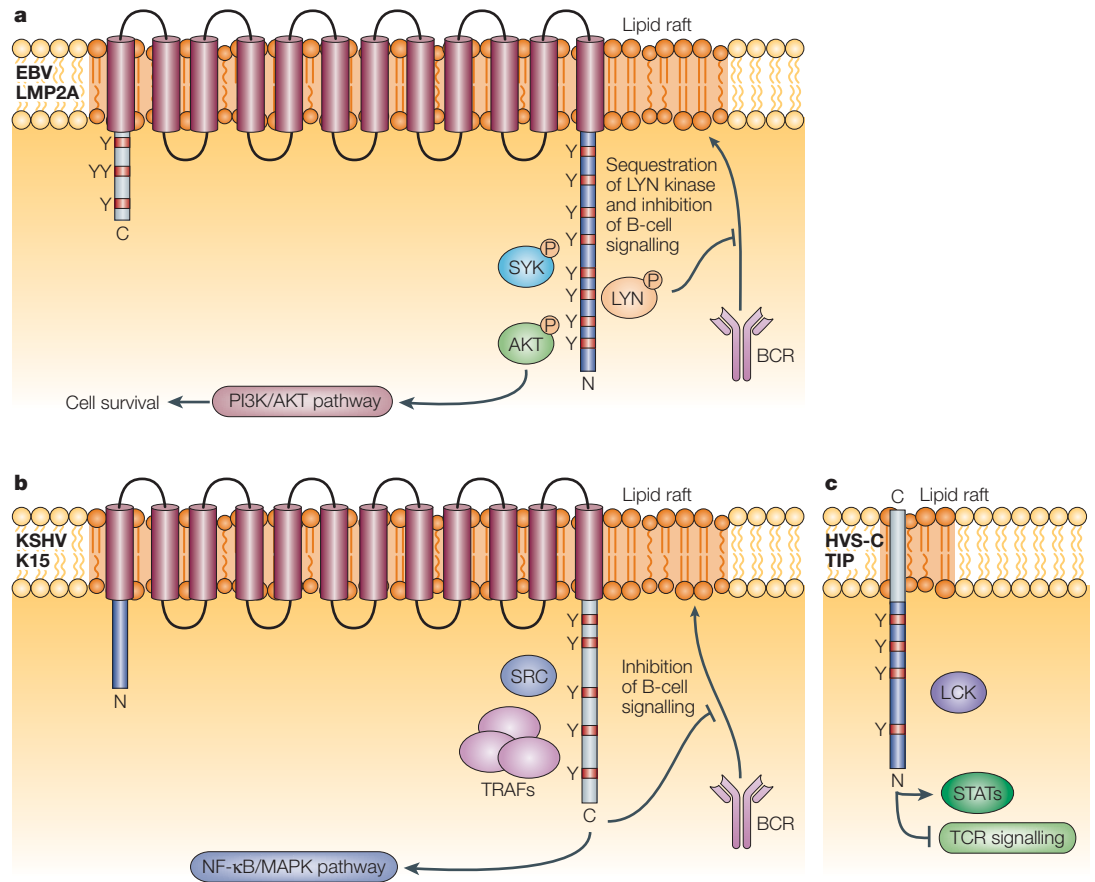
**HVS TIP.** The ORF that encodes TIP is only present in the genome of HVS subgroup C. The TIP protein contains an N-terminal glutamate-rich region, a serine-rich region, a kinase-interacting domain and a C-terminal hydrophobic tail. TIP can associate with the major T-cell tyrosine kinase LCK<sup>63</sup> (FIG. 4). TIP interacts with LCK through the C-termini of its SRC-family kinase

(CSKH) motif and its SH3-binding motif<sup>64,65</sup>. Although some reports have suggested that interaction of TIP with LCK leads to its activation *in vitro* and *in vivo*<sup>66</sup>, other reports suggest that stable and low expression levels of TIP downregulate the TCR-mediated signal-transduction pathway<sup>65,67,68</sup>. Therefore, the exact role of TIP in the modulation of the TCR signalling pathway remains controversial. TIP-mediated activation of LCK was also shown to lead to the activation of signal transducers and activators of transcription (STATs), which are thought to have an important role in the transformation process<sup>69</sup>. So, paralleling the case of EBV LMP2A and KSHV K15 with the BCR signalling pathway, HVS TIP seems to modulate TCR signalling pathways and its role in the transformation process might depend on its expression level and, therefore, its ability to activate LCK. However, as the deletion of the *tip* gene in the genome of HVS subgroup C renders the mutant virus incapable of immortalizing primary T cells<sup>42</sup> and HVS TIP-transgenic animals develop T-cell lymphomas<sup>70</sup>, it is generally accepted that TIP is required for HVS-induced transformation. The two transforming proteins of HVS subgroup C, STP and TIP, probably function together to transform HVS-infected cells.

#### Other transforming proteins of $\gamma$ -herpesviruses

In addition to the first and last ORFs of EBV and KSHV, many other genes of these  $\gamma$ -herpesviruses modulate cell signalling and could therefore be involved in the neoplastic disease process. These include additional viral signalling proteins as well as transcription factors that are encoded by the virus.

**EBV EBNA2.** Many of the latent EBV proteins have important roles in cellular transformation. EBV nuclear antigen 2 (EBNA2) contains a polyproline stretch, an 18-residue Arg–Gly repeat, and an acidic C-terminus. It is a promiscuous transcriptional activator, activating the



**Figure 4 | Cellular effects of the signal modulatory proteins EBV LMP2A, KSHV K15 and HVS TIP.** These proteins inhibit BCR and TCR signal transduction and help to maintain viral latency. **a** | EBV LMP2A contains multiple tyrosine residues (Y) which can interact with SYK and LYN. The latter interaction sequesters LYN from the BCR complex, resulting in inhibition of B-cell signalling pathways. The PI3K and AKT pathways are also activated, leading to cell survival. **b** | KSHV K15 can activate SRC and MAPK and associate with TRAFs. These interactions appear to dampen BCR signalling. **c** | HVS TIP, which is only encoded by subgroup C viruses, can interact with LCK and inhibit T-cell signalling. BCR, B-cell receptor; EBV, Epstein–Barr virus; HVS, herpesvirus saimiri; KSHV, Kaposi’s sarcoma-associated virus; LMP, latent membrane protein; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; STAT, signal transducers and activators of transcription; TCR, T-cell receptor; TIP, tyrosine-kinase-interacting protein; TRAF, tumour-necrosis-factor-receptor-associated factor.

promoters of both viral and cellular genes<sup>71–73</sup> through its interaction with a range of cellular transcription factors<sup>72,74,75</sup>. EBNA2 seems to be essential for B-cell transformation as deletion of the gene encoding EBNA2 from wild-type EBV renders the mutant virus incapable of immortalizing B lymphocytes<sup>76–78</sup>. Therefore, the ability of EBNA2 to upregulate the transcription of both cellular and viral genes is likely to contribute to the transforming mechanism of EBV.

**EBV EBNA3s.** The genes that encode EBNA3A, -3B and -3C are located in a tandem array in the EBV viral genome (FIG. 2). EBNA3A and -3C are essential for B-cell transformation<sup>79,80</sup>, whereas -3B has been shown to be dispensable<sup>81</sup>. The three EBNA3 nuclear proteins are hydrophilic and share a common N-terminal domain but have different C-termini. All three EBNA3 proteins can interfere with EBNA2 activation by disrupting its interaction with the DNA-binding protein RBP-Jκ, thereby suppressing EBNA2-mediated

transactivation<sup>82–84</sup>. EBNA3C has been shown to cooperate with the proto-oncogene *RAS* to immortalize and transform rodent fibroblasts<sup>85</sup> and can directly interact with the retinoblastoma (Rb) tumour-suppressor protein, rendering it inactive and promoting tumour progression<sup>85</sup>. So, EBNA3C can promote cellular proliferation and override the G1–S-phase cell-cycle checkpoint, and can cooperate with EBNA2 and -3A to modulate cellular gene expression in EBV-infected lymphocytes.

**KSHV Kaposin.** Kaposin is expressed as a latent transcript in KS and PEL cells<sup>86</sup> and is also induced during the lytic cycle. There are several protein isoforms of Kaposin — designated as A, B and C<sup>87</sup>. The smallest isoform (Kaposin A) has a molecular mass of 6 kDa, and has transforming potential in nude mice and in a fibroblast-transformation assay<sup>88–90</sup>. The activity of Kaposin A seems to be mediated through its direct interaction with cytohesin-1, a guanine

nucleotide-exchange factor for ARF GTPases that regulates integrin-mediated cell adhesion<sup>88</sup>.

**KSHV vGPCR.** KSHV vGPCR is a potent signalling protein, which has greatest sequence similarity to the cellular IL-8 receptor<sup>91,92</sup> and is expressed in some KS, MCD and PEL tumour cells<sup>93</sup>. vGPCR is a seven-transmembrane receptor protein that is constitutively active, similar to EBV LMP1 and KSHV K1 (REF. 94). Although it does not require ligand binding for activation, it binds both the CXC and CC families of chemokines<sup>94,95</sup>. vGPCR signalling is positively enhanced by binding to CXC chemokines such as GRO- $\alpha$  and IL-8 (REF. 95), but is inhibited by binding to the CXC chemokines interferon (IFN)- $\gamma$ -inducible 10-kDa protein (IP-10) and stromal cell-derived factor 1 $\alpha$  (SDF-1 $\alpha$ )<sup>96,97</sup>. Other CC chemokines, such as RANTES and macrophage inflammatory protein 1 $\alpha$  (MIP-1 $\alpha$ ), are able to bind vGPCR, but do not affect its signalling potential. KSHV vGPCR can activate both the MAPK and PI3K pathways, leading to the activation of many cell-signalling networks<sup>98,99</sup>. The pleiotropic effects of KSHV vGPCR on cell-signalling pathways in epithelial, fibroblast, endothelial and B cells contributes to the increased transcription of cellular genes and paracrine factors that are involved in cell proliferation, immortalization and transformation<sup>99–103</sup>. Indeed, expression of the KSHV vGPCR gene in rat kidney cells and NIH 3T3 fibroblasts induced morphological changes and foci formation<sup>94,102</sup>. vGPCR has been shown to immortalize endothelial cells and promote tumour formation in cells expressing KSHV latent genes, indicating that the KSHV latent and lytic viral genes function cooperatively in the promotion of Kaposi's sarcomagenesis<sup>101,104</sup>. Induction of transformation by vGPCR is associated with an increased secretion of VEGF and VEGF receptor 2 (VEGFR-2) that leads to the induction of an angiogenic response *in vitro* and *in vivo*<sup>98,102,101</sup>. Furthermore, transgenic mice expressing vGPCR have morphological features that are similar to KS<sup>104–106</sup>. So, the EBV LMP1, KSHV K1 and KSHV vGPCR transforming proteins seem to promote tumour progression and angiogenesis through a paracrine mechanism that involves the upregulation and secretion of angiogenic factors.

#### Proteins essential for episomal maintenance

The latent viral proteins EBV EBNA1, KSHV LANA and HVS ORF73 are essential for episomal maintenance of the viral genome (TABLE 3). These three proteins either contribute indirectly to the immortalization process by maintaining the viral latent state, or directly contribute to tumorigenesis by a more active mechanism.

**EBV EBNA1.** The EBNA1 protein is a sequence-specific DNA-binding protein that binds to the EBV origin of replication (*oriP*) and is essential for the maintenance of the viral episome, as well as for the initiation of latent viral replication. Several observations indicate that EBNA1 might also contribute to EBV transformation: transgenic mice expressing EBNA1 in the B-lymphocyte compartment develop B-cell lymphomas<sup>107</sup>; an

EBNA1-deleted virus was reduced in its ability to immortalize B cells<sup>108</sup>; and expression of EBNA1 can enhance the tumorigenicity of EBV-negative NPC epithelial cells<sup>109</sup>. This property of EBNA1 might be linked to its ability to induce expression of Bcl-x<sub>L</sub><sup>110</sup>. However, it should be noted that other research groups have reported that epithelial expression of EBNA1 results in cell cytotoxicity<sup>111</sup>.

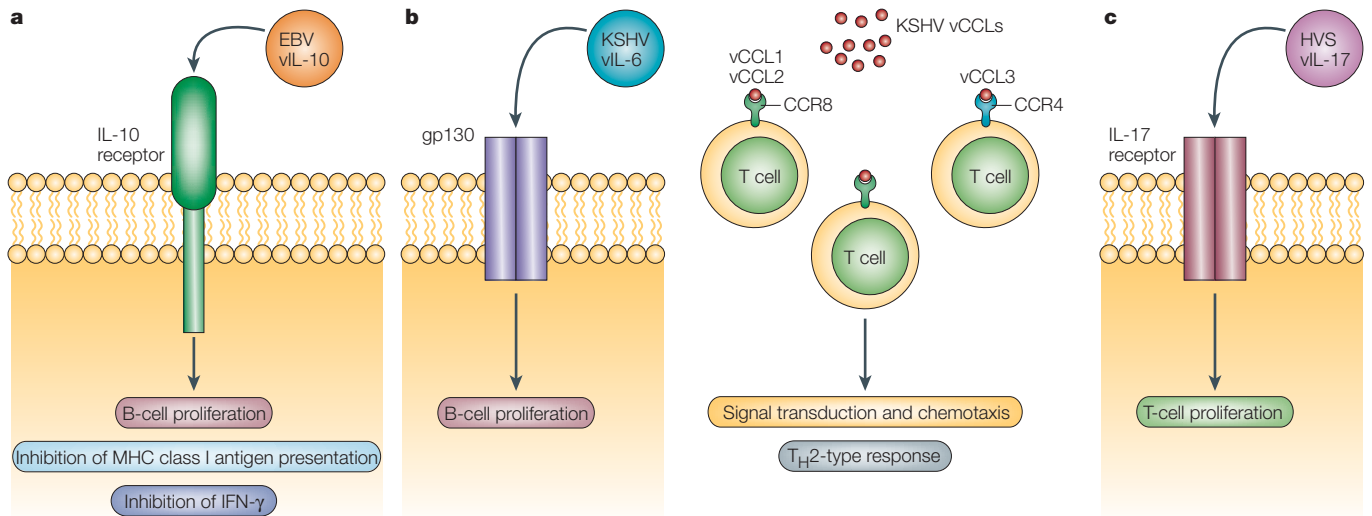
EBNA1 also functions as an immunomodulatory protein. It contains a Gly–Ala repeat region, the size of which varies among different viral isolates. This repeat region hinders antigen processing by the ubiquitin-mediated proteasomal pathway and thereby inhibits MHC class I antigen presentation<sup>112,113</sup>. The end result is the inhibition of the cytotoxic T cell (CTL)-mediated response against EBV-infected B cells (FIG. 5). So, not only is EBNA1 crucial for EBV latency and latent replication, but it also helps the virus evade host immune detection.

**KSHV LANA.** Similar to EBNA1, KSHV LANA is a nuclear phosphoprotein that is essential for episome maintenance and segregation. LANA is expressed during latency in KS, MCD and PEL cells<sup>114–116</sup> (TABLE 2), binds the KSHV terminal-repeat (TR) regions and tethers the viral episome to the host chromosome<sup>117–120</sup>. Similar to EBNA1, LANA contains a central repeat region, which is also variable in length but is composed of acidic amino acids. Like many other cellular proteins, LANA has been shown to bind and inactivate the tumour-suppressor functions of the Rb and p53 proteins<sup>121,122</sup>. Furthermore, LANA can upregulate expression of  $\beta$ -catenin, and stabilize its expression by sequestering its inhibitor, glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ )<sup>123</sup>. The ability to target GSK-3 $\beta$  is necessary for LANA to stimulate entry into S-phase, a function that might contribute to KSHV-associated neoplasia. So, in addition to being the guardian of KSHV latency, LANA seems to also modulate several cell-cycle pathways.

**HVS ORF73.** Analogous to EBV EBNA1 and KSHV LANA, the HVS ORF73 protein is a nuclear protein and can bind to both host chromosomes and the HVS terminal-repeat region<sup>124–127</sup>. ORF73 from HVS subgroup A11 can synergize with the transcription factor p32 to activate the ORF73 promoter element but not the HVS ORF50 gene promoter<sup>128</sup>. By contrast, the HVS C488 ORF73 protein can downregulate the HVS ORF50 promoter and inhibit viral replication of HVS in owl-monkey kidney cells<sup>125</sup>. The HVS *orf50* gene (also called *rta*) codes for an important viral transcription factor that is essential for HVS replication. So, similar to KSHV LANA, the ORF73 protein of HVS subgroup C preserves viral latency in the host by inhibiting lytic replication<sup>125,129</sup>.

#### Viral cytokines

The genomes of EBV, KSHV and HVS encode one or more viral cytokines (TABLE 3), the genes of which have been obtained through molecular piracy of the host genome. These viral cytokines have distinct homology to their cellular counterparts and can modulate cell



**Figure 5 | Viral cytokines and chemokines encoded by EBV, KSHV and HVS.** **a** | The EBV vIL-10 cytokine can support B-cell proliferation and inhibit MHC class I antigen presentation. **b** | The KSHV vIL-6 cytokine promotes B-cell proliferation by directly interacting with gp130 and the KSHV vCCLs seem to direct inflammatory cell recruitment towards a  $T_H2$ -type response. **c** | The HVS vIL-17 cytokine can promote T-cell proliferation. EBV, Epstein-Barr virus; KSHV, Kaposi's sarcoma-associated herpesvirus; HVS, herpesvirus saimiri; IL, interleukin; MHC, major histocompatibility complex.

pathways to promote survival of the infected cell and escape from host immune surveillance.

**EBV IL-10.** The EBV BCRF1 gene codes for a viral IL-10 (vIL-10) cytokine that is homologous to human IL-10, and is expressed after the initial primary infection of B cells, as well as late in the lytic cycle<sup>130</sup>. EBV vIL-10 has been shown to downregulate the expression of transporter protein 1 associated with MHC class I antigen presentation<sup>131</sup> and to prevent monocytes and macrophages from activating T cells<sup>132,133</sup>. Other studies have reported that EBV vIL-10 is necessary for EBV-mediated B-cell transformation<sup>130,134,135</sup> and can enhance survival of EBV-infected B cells by blocking the IFN- $\gamma$  response<sup>136,137</sup> (FIG. 5). EBV vIL-10 seems to block IFN synthesis during primary or reactivated EBV infection, and might contribute indirectly to transformation by allowing the EBV-infected cell to survive the host response.

**KSHV vIL-6.** KSHV also encodes a viral interleukin, vIL-6, which shows homology to cellular IL-6. KSHV vIL-6 is secreted from KSHV-positive B cells and can support proliferation of B lymphocytes and IL-6-dependent mouse myeloma cell lines<sup>138-140</sup>. vIL-6 has also been shown to induce secretion of human IL-6 and to support the growth of PEL cells<sup>141,142</sup>. In contrast to cellular IL-6, vIL-6 protects KSHV-infected PEL cells from the antiviral effects of IFN- $\alpha$ <sup>143</sup>. In addition, IFN- $\alpha$  can activate expression of vIL-6 through the IFN-stimulated response elements (ISREs) in the vIL-6 promoter<sup>143</sup>, thereby enhancing virus survival. Despite their similarity in sequence and function, cellular IL-6 and vIL-6 display differences in receptor dependence. Although cellular IL-6 requires both IL-6 receptor- $\alpha$  and the gp130 subunits,

vIL-6 seems to require only gp130 (REF. 144) (FIG. 5), demonstrating that the viral protein has successfully subverted host-cell control. In the context of KSHV disease, vIL-6 is expressed in PELs and is also highly expressed in MCD, where it seems to contribute to progression of this disease<sup>145-147</sup> (TABLE 2). By contrast, vIL-6 is expressed at low levels in a small fraction of KS cells<sup>86</sup>. So, vIL-6 is a multifunctional cytokine that contributes to KSHV-associated disease progression by continuously stimulating the IL-6-receptor signalling pathway and rescuing virus-infected cells from programmed cell death

**KSHV vMIPs/vCCLs.** KSHV encodes several viral gene products that are homologous to MIPs and are also known as vCCLs. KSHV ORFs K6 (vMIP-1/vMIP-1a/vCCL-1), K4 (vMIP-II/vMIP-1b/vCCL-2) and K4.1 (v-MIP-III/vBCK/vCCL3) encode chemokines with homology to cellular CC chemokines such as MIP-1 $\alpha$  and RANTES. Unlike cellular MIP-1 $\alpha$ , vMIPs I and II bind efficiently to the chemokine receptor CCR8 (REFS 148,149) as agonists, whereas the receptor activated by vMIP III is CCR4 (REF. 150). These viral MIPs have also been shown to induce signal transduction and chemotaxis in monocytes<sup>151</sup> and, in contrast to cellular MIP-1 $\alpha$  and RANTES, these proteins are highly angiogenic in a chorioallantoic assay, a technique that uses chick embryo membranes to measure vascularization by exogenous factors<sup>148</sup>. An immunomodulatory role for vMIP-II and vMIP-III has also been proposed in directing inflammatory cell recruitment away from a  $T_H1$ -type response towards a  $T_H2$ -type response, thereby facilitating evasion from host cytotoxic T cells<sup>148,152</sup> (FIG. 5). So, these multiple viral MIPs could contribute to KS pathogenesis by inducing inflammatory infiltration and angioperplasia.

**HVS vIL-17.** HVS also encodes a viral interleukin, vIL-17, which has been shown to support T-cell proliferation<sup>153</sup>. Recombinant vIL-17 was shown to activate NF- $\kappa$ B and promote IL-6 secretion in fibroblasts<sup>153</sup> (FIG. 5). Interestingly, it was the discovery of vIL-17 that led to the identification of cellular IL-17 and the IL-17 receptor<sup>153,154</sup>.

### Viral cyclins

Although EBV seems to upregulate the expression of the cellular cyclins E and D2 100-fold<sup>155</sup>, ORFs 72 of both KSHV and HVS encode a homologue of cellular cyclin D<sup>91,156</sup>. Surprisingly, neither of these viral cyclins alone have been found to possess transforming potential. Additionally, expression of the KSHV viral cyclin induced cellular apoptosis<sup>157</sup>, indicating that high expression levels of this protein are toxic to the cell. Interestingly, the KSHV vCyclin, which is expressed during viral latency, can induce B-cell lymphomas in p53-deficient transgenic mice<sup>158</sup> and therefore might be oncogenic in the absence of the apoptosis-inducing tumour-suppressor protein p53. In HVS, ORF72 is expressed in HVS-immortalized common marmoset T lymphocytes<sup>159</sup>. However, deletion of ORF72 did not impair the ability of the mutant virus to replicate or to induce T-cell immortalization and disease in common marmosets<sup>160</sup>. The evidence indicates that the viral cyclins are likely to have an auxiliary or accessory role in viral tumorigenesis.

In addition to the viral genes listed above, the  $\gamma$ -herpesviruses genomes also contain genes that are involved in immune evasion and the prevention of apoptosis, which are not discussed here due to space restrictions. These immune evasion and anti-apoptotic  $\gamma$ -herpesvirus proteins are thought to promote virus survival and ensure a lifelong period of viral persistence in the host.

### Summary

Historically, research on the tumour virology of both RNA and DNA viruses has given rise to important concepts in cell biology, including the discovery of cellular proto-oncogenes and tumour suppressors. These findings paved the way for the current elucidation of mechanisms by which non-viral cancers arise. So, the study of tumour viruses has been instrumental to our present knowledge of the neoplastic process, be it virus-related or not.

Many tumour viruses stimulate proliferation of the infected cell, and the analysis of viral genes associated with transformation has revealed many different strategies by which viruses can deregulate cell growth. In common with other DNA tumour viruses such as polyomaviruses and papillomaviruses, the  $\gamma$ -herpesviruses EBV, KSHV and HVS are associated with several different malignancies in the natural or experimental host. One conundrum is that, although EBV and KSHV encode a large number of transforming genes, EBV- and KSHV-associated malignancies are often (but not always) seen in the context of immune suppression, such as HIV co-infection or iatrogenic immune-suppression. This suggests that the normal host immune surveillance mechanisms in healthy individuals are generally able to keep these viruses in check. Furthermore, as tumorigenesis is a multi-step process, infection with EBV and/or KSHV is probably one of many events that leads to the onset of human cancer.

As described above, KSHV, EBV and HVS encode a diverse array of viral genes that contribute to the neoplastic process. By encoding unique viral proteins, viral homologues of cellular proteins or proteins involved in immune evasion, or by activating a plethora of cellular genes, these viruses have evolved strategies for deregulating and perturbing normal cellular pathways that would otherwise lead to apoptosis, activation of the host immune system and an arrest in cell growth. Although some of these viral genes do not possess oncogenic potential by themselves, they might have an ancillary role in tumorigenesis by augmenting the function of another viral gene, or they might have tumorigenic potential in the context of a mutant cytogenetic background. Furthermore, as the  $\gamma$ -herpesviruses have multiple tropisms for different cell types, certain subsets of viral genes might help support virus survival in different cellular environments, ensuring a lifelong persistence in the host. So, although the individual genes of these  $\gamma$ -herpesviruses may or may not appear congruent, the strategies that are used to subvert cell pathways and override host cell-cycle checkpoints are often concordant, and support the maintenance of viral latency with intermittent periods of productive viral replication in the host. In conclusion, it can be surmised that the sum of the functions of all these  $\gamma$ -herpesvirus proteins probably contributes to the progression and development of neoplastic disease in the infected host at opportune times.

1. Damania, B.  *$\gamma$ -Herpesviruses of Non-Human Primates. The Human Herpesviruses: Biology, Therapy and Immunoprophylaxis* (Cambridge University Press, 2004).
2. Epstein, M. A., Achong, B. & Barr, Y. Virus particles in culture lymphoblasts from Burkitt's lymphoma. *Lancet* **15**, 702–703 (1964).  
**First report of the identification of EBV viruses in B cells from Burkitt's lymphoma patients.**
3. Melendez, L. V., Daniel, M. D., Hunt, R. D. & Garcia, F. G. An apparently new herpesvirus from primary kidney cultures of the squirrel monkey (*Saimiri sciureus*). *Lab. Anim. Care* **18**, 374–381 (1968).  
**Identification of a novel herpesvirus in the squirrel monkey.**
4. Chang, Y. *et al.* Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* **266**, 1865–1869 (1994).

### Original paper reporting the discovery of the first human rhadinovirus, KSHV.

5. Fleckenstein, B. *et al.* Tumour induction with DNA of oncogenic primate herpesviruses. *Nature* **274**, 57–59 (1978).  
**First report to show that an intramuscular injection of purified virion HVS causes malignant disease in the experimental host.**
6. Wang, D., Liebowitz, D. & Kieff, E. An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. *Cell* **43**, 831–840 (1985).  
**Showed that the main oncoprotein of EBV, LMP1, could transform cells *in vitro*.**
7. Dawson, C. W., Rickinson, A. B. & Young, L. S. Epstein-Barr virus latent membrane protein inhibits human epithelial cell differentiation. *Nature* **344**, 777–780 (1990).

8. Kaye, K. M., Izumi, K. M. & Kieff, E. Epstein-Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation. *Proc. Natl Acad. Sci. USA* **90**, 9150–9154 (1993).  
**First report to show that the LMP1 viral protein is essential for B-cell immortalization.**
9. Uchida, J. *et al.* Mimicry of CD40 signals by Epstein-Barr virus LMP1 in B-lymphocyte responses. *Science* **286**, 300–303 (1999).
10. Gires, O. *et al.* Latent membrane protein 1 of Epstein-Barr virus mimics a constitutively active receptor molecule. *EMBO J.* **16**, 6131–6140 (1997).
11. Hatzivassiliou, E., Miller, W. E., Raab-Traub, N., Kieff, E. & Mosialos, G. A fusion of the EBV latent membrane protein-1 (LMP1) transmembrane domains to the CD40 cytoplasmic domain is similar to LMP1 in constitutive activation of epidermal growth factor receptor expression, nuclear

- factor- $\kappa$ B, and stress-activated protein kinase. *J. Immunol.* **160**, 1116–1121 (1998).
12. Kilger, E., Kieser, A., Baumann, M. & Hammerschmidt, W. Epstein–Barr virus-mediated B-cell proliferation is dependent upon latent membrane protein 1, which simulates an activated CD40 receptor. *EMBO J.* **17**, 1700–1709 (1998).
  13. Huen, D. S., Henderson, S. A., Croom-Carter, D. & Rowe, M. The Epstein–Barr virus latent membrane protein-1 (LMP1) mediates activation of NF- $\kappa$ B and cell surface phenotype via two effector regions in its carboxy-terminal cytoplasmic domain. *Oncogene* **10**, 549–560 (1995).
  14. Paine, E., Scheinman, R. I., Baldwin, A. S. Jr & Raab-Traub, N. Expression of LMP1 in epithelial cells leads to the activation of a select subset of NF- $\kappa$ B/Rel family proteins. *J. Virol.* **69**, 4572–4576 (1995).
  15. Kaye, K. M. *et al.* An Epstein–Barr virus that expresses only the first 231 LMP1 amino acids efficiently initiates primary B-lymphocyte growth transformation. *J. Virol.* **73**, 10525–10530 (1999).
  16. Devergne, O. *et al.* Role of the TRAF-binding site and NF- $\kappa$ B activation in Epstein–Barr virus latent membrane protein 1-induced cell gene expression. *J. Virol.* **72**, 7900–7908 (1998).
  17. Eliopoulos, A. G., Blake, S. M., Floettmann, J. E., Rowe, M. & Young, L. S. Epstein–Barr virus-encoded latent membrane protein 1 activates the JNK pathway through its extreme C terminus via a mechanism involving TRADD and TRAF2. *J. Virol.* **73**, 1023–1035 (1999).
  18. Izumi, K. M. *et al.* The Epstein–Barr virus oncoprotein latent membrane protein 1 engages the tumor necrosis factor receptor-associated proteins TRADD and receptor-interacting protein (RIP) but does not induce apoptosis or require RIP for NF- $\kappa$ B activation. *Mol. Cell. Biol.* **19**, 5759–5767 (1999).
  19. Sandberg, M., Hammerschmidt, W. & Sugden, B. Characterization of LMP-1's association with TRAF1, TRAF2, and TRAF3. *J. Virol.* **71**, 4649–4656 (1997).
  20. Rothe, M., Wong, S. C., Henzel, W. J. & Goeddel, D. V. A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. *Cell* **78**, 681–692 (1994).
- First report identifying the presence of TNF-receptor-associated proteins (TRAFs).**
21. Izumi, K. M. *et al.* The residues between the two transformation effector sites of Epstein–Barr virus latent membrane protein 1 are not critical for B-lymphocyte growth transformation. *J. Virol.* **73**, 9908–9916 (1999).
  22. Dawson, C. W., Tramontanis, G., Eliopoulos, A. G. & Young, L. S. Epstein–Barr virus latent membrane protein 1 (LMP1) activates the phosphatidylinositol 3-kinase/Akt pathway to promote cell survival and induce actin filament remodeling. *J. Biol. Chem.* **278**, 3694–3704 (2003).
  23. Eliopoulos, A. G. & Young, L. S. Activation of the cJun N-terminal kinase (JNK) pathway by the Epstein–Barr virus-encoded latent membrane protein 1 (LMP1). *Oncogene* **16**, 1731–1742 (1998).
  24. Higuchi, M., Izumi, K. M. & Kieff, E. Epstein–Barr virus latent-infection membrane proteins are palmitoylated and raft-associated: protein 1 binds to the cytoskeleton through TNF receptor cytoplasmic factors. *Proc. Natl Acad. Sci. USA* **98**, 4675–4680 (2001).
  25. Yasui, T., Luftig, M., Soni, V. & Kieff, E. Latent infection membrane protein transmembrane FWLY is critical for intermolecular interaction, raft localization, and signaling. *Proc. Natl Acad. Sci. USA* **101**, 278–283 (2004).
  26. Muroso, S. *et al.* Induction of cyclooxygenase-2 by Epstein–Barr virus latent membrane protein 1 is involved in vascular endothelial growth factor production in nasopharyngeal carcinoma cells. *Proc. Natl Acad. Sci. USA* **98**, 6905–6910 (2001).
  27. Wakisaka, N., Muroso, S., Yoshizaki, T., Furukawa, M. & Pagano, J. S. Epstein–Barr virus latent membrane protein 1 induces and causes release of fibroblast growth factor-2. *Cancer Res.* **62**, 6337–6344 (2002).
  28. Yoshizaki, T., Sato, H., Furukawa, M. & Pagano, J. S. The expression of matrix metalloproteinase 9 is enhanced by Epstein–Barr virus latent membrane protein 1. *Proc. Natl Acad. Sci. USA* **95**, 3621–3626 (1998).
  29. Thornburg, N. J., Pathmanathan, R. & Raab-Traub, N. Activation of nuclear factor- $\kappa$ B p50 homodimer/Bcl-3 complexes in nasopharyngeal carcinoma. *Cancer Res.* **63**, 8293–8301 (2003).
  30. Miller, W. E., Cheshire, J. L., Baldwin, A. S. Jr & Raab-Traub, N. The NPC derived C15 LMP1 protein confers enhanced activation of NF- $\kappa$ B and induction of the EGFR in epithelial cells. *Oncogene* **16**, 1869–1877 (1998).
  31. Lee, H. *et al.* Deregulation of cell growth by the K1 gene of Kaposi's sarcoma-associated herpesvirus. *Nature Med.* **4**, 435–440 (1998).
  32. Prakash, O. *et al.* Tumorigenesis and aberrant signaling in transgenic mice expressing the human herpesvirus-8 K1 gene. *J. Natl Cancer Inst.* **94**, 926–935 (2002).
  33. Lagunoff, M. & Ganem, D. The structure and coding organization of the genomic termini of Kaposi's sarcoma-associated herpesvirus. *Virology* **236**, 147–154 (1997).
  34. Lagunoff, M., Majeti, R., Weiss, A. & Ganem, D. Deregulated signal transduction by the K1 gene product of Kaposi's sarcoma-associated herpesvirus. *Proc. Natl Acad. Sci. USA* **96**, 5704–5709 (1999).
  35. Lee, H. *et al.* Identification of an immunoreceptor tyrosine-based activation motif of K1 transforming protein of Kaposi's sarcoma-associated herpesvirus. *Mol. Cell. Biol.* **18**, 5219–5228 (1998).
  36. Tomlinson, C. C. & Damania, B. The K1 protein of Kaposi's sarcoma-associated herpesvirus activates the Akt signaling pathway. *J. Virol.* **78**, 1918–1927 (2004).
  37. Samaniego, F., Markham, P. D., Gallo, R. C. & Ensign, B. Inflammatory cytokines induce AIDS-Kaposi's sarcoma-derived spindle cells to produce and release basic fibroblast growth factor and enhance Kaposi's sarcoma-like lesion formation in nude mice. *J. Immunol.* **154**, 3582–3592 (1995).
  38. Samaniego, F., Pati, S., Karp, J., Prakash, O. & Bose, D. Human herpesvirus 8 K1-associated nuclear factor- $\kappa$ B-dependent promoter activity: role in Kaposi's sarcoma inflammation? *J. Natl Cancer Inst. Monogr.* **28**, 15–23 (2001).
  39. Lee, B. S., Conrole, M., Tang, Z., Harris, N. L. & Jung, J. U. Structural analysis of the Kaposi's sarcoma-associated herpesvirus K1 protein. *J. Virol.* **77**, 8072–8086 (2003).
  40. Wang, L. *et al.* The Kaposi's sarcoma-associated herpesvirus (KSHV/HHV8) K1 protein induces expression of angiogenic and invasion factors. *Cancer Res.* **64**, 2774–2781 (2004).
  41. Desrosiers, R. C. & Falk, L. A. Herpesvirus saimiri strain variability. *J. Virol.* **43**, 352–356 (1982).
  42. Duboise, S. M., Guo, J., Czajak, S., Desrosiers, R. C. & Jung, J. U. STP and TIP are essential for herpesvirus saimiri oncogenicity. *J. Virol.* **72**, 1308–1313 (1998).
  43. Murthy, S. C., Trimble, J. J. & Desrosiers, R. C. Deletion mutants of herpesvirus saimiri define an open reading frame necessary for transformation. *J. Virol.* **63**, 3307–3314 (1989).
  44. Jung, J. U. *et al.* Identification of transforming genes of subgroup A and C strains of herpesvirus saimiri. *Proc. Natl Acad. Sci. USA* **88**, 7051–7055 (1991).
- Demonstrated that the stp gene of HVS subgroup C was more oncogenic than the stp gene of subgroup A.**
45. Choi, J. K., Ishido, S. & Jung, J. U. The collagen repeat sequence is a determinant of the degree of herpesvirus saimiri STP transforming activity. *J. Virol.* **74**, 8102–8110 (2000).
  46. Jung, J. U. & Desrosiers, R. C. Association of the viral oncoprotein STP-C488 with cellular Ras. *Mol. Cell. Biol.* **15**, 6506–6512 (1995).
  47. Lee, H. *et al.* Role of cellular tumor necrosis factor receptor-associated factors in NF- $\kappa$ B activation and lymphocyte transformation by herpesvirus saimiri STP. *J. Virol.* **73**, 3913–3919 (1999).
  48. Bussou, P. *et al.* Consistent transcription of the Epstein–Barr virus LMP2 gene in nasopharyngeal carcinoma. *J. Virol.* **66**, 3257–3262 (1992).
  49. Sample, J., Liebowitz, D. & Kieff, E. Two related Epstein–Barr virus membrane proteins are encoded by separate genes. *J. Virol.* **63**, 933–937 (1989).
  50. Fruhling, S. & Longnecker, R. The immunoreceptor tyrosine-based activation motif of Epstein–Barr virus LMP2A is essential for blocking BCR-mediated signal transduction. *Virology* **235**, 241–251 (1997).
  51. Miller, C. L., Longnecker, R. & Kieff, E. Epstein–Barr virus latent membrane protein 2A blocks calcium mobilization in B lymphocytes. *J. Virol.* **67**, 3087–3094 (1993).
  52. Dykstra, M. L., Longnecker, R. & Pierce, S. K. Epstein–Barr virus co-opts lipid rafts to block the signaling and antigen transport functions of the BCR. *Immunity* **14**, 57–67 (2001).
  53. Miller, C. L., Lee, J. H., Kieff, E. & Longnecker, R. An integral membrane protein (LMP2) blocks reactivation of Epstein–Barr virus from latency following surface immunoglobulin crosslinking. *Proc. Natl Acad. Sci. USA* **91**, 772–776 (1994).
  54. Fukuda, M. & Longnecker, R. Latent membrane protein 2A inhibits transforming growth factor- $\beta$ 1-induced apoptosis through the phosphatidylinositol 3-kinase/Akt pathway. *J. Virol.* **78**, 1697–1705 (2004).
  55. Caldwell, R. G., Wilson, J. B., Anderson, S. J. & Longnecker, R. Epstein–Barr virus LMP2A drives B cell development and survival in the absence of normal B cell receptor signals. *Immunity* **9**, 405–411 (1998).
  56. Longnecker, R., Miller, C. L., Miao, X. Q., Tomkinson, B. & Kieff, E. The last seven transmembrane and carboxy-terminal cytoplasmic domains of Epstein–Barr virus latent membrane protein 2 (LMP2) are dispensable for lymphocyte infection and growth transformation *in vitro*. *J. Virol.* **67**, 2006–2013 (1993).
  57. Scholle, F., Benoit, K. M. & Raab-Traub, N. Epstein–Barr virus LMP2A transforms epithelial cells, inhibits cell differentiation, and activates Akt. *J. Virol.* **74**, 10681–10689 (2000).
  58. Morrison, J. A., Klingelutz, A. J. & Raab-Traub, N. Epstein–Barr virus latent membrane protein 2A activates  $\beta$ -catenin signaling in epithelial cells. *J. Virol.* **77**, 12276–12284 (2003).
  59. Glenn, M., Rainbow, L., Aurd, F., Davison, A. & Schulz, T. F. Identification of a spliced gene from Kaposi's sarcoma-associated herpesvirus encoding a protein with similarities to latent membrane proteins 1 and 2A of Epstein–Barr virus. *J. Virol.* **73**, 6953–6963 (1999).
  60. Choi, J., Lee, B. S., Shim, S., Li, M. & Jung, J. U. Identification of the novel K15 gene at the right-most end of Kaposi's sarcoma-associated herpesvirus genome. *J. Virol.* **74**, 436–446 (2000).
  61. Poole, L. J. *et al.* Comparison of genetic variability at multiple loci across the genomes of the major subtypes of Kaposi's sarcoma-associated herpesvirus reveals evidence for recombination and for two distinct types of open reading frame K15 alleles at the right-hand end. *J. Virol.* **73**, 6646–6660 (1999).
  62. Brinkmann, M. M. *et al.* Activation of mitogen-activated protein kinase and NF- $\kappa$ B pathways by a Kaposi's sarcoma-associated herpesvirus K15 membrane protein. *J. Virol.* **77**, 9346–9358 (2003).
  63. Biesinger, B. *et al.* The product of the herpesvirus saimiri open reading frame 1 (TIP) interacts with T-cell-specific kinase p56lck in transformed cells. *J. Biol. Chem.* **270**, 4729–4734 (1995).
  64. Jung, J. U. *et al.* Identification of Lck-binding elements in tip of herpesvirus saimiri. *J. Biol. Chem.* **270**, 20660–20667 (1995).
  65. Jung, J. U. *et al.* Downregulation of Lck-mediated signal transduction by TIP of herpesvirus saimiri. *J. Virol.* **69**, 7814–7822 (1995).
  66. Kjellen, P., Amdjadi, K., Lund, T. C., Medveczky, P. G. & Sefton, B. M. The herpesvirus saimiri TIP484 and TIP488 proteins both stimulate lck tyrosine protein kinase activity *in vivo* and *in vitro*. *Virology* **297**, 281–288 (2002).
  67. Lund, T., Medveczky, M. M. & Medveczky, P. G. Herpesvirus saimiri TIP-484 membrane protein markedly increases p56lck activity in T cells. *J. Virol.* **71**, 378–382 (1997).
  68. Guo, J. *et al.* Enhanced downregulation of Lck-mediated signal transduction by a Y114 mutation of herpesvirus saimiri TIP. *J. Virol.* **71**, 7092–7096 (1997).
  69. Lund, T. C., Prator, P. C., Medveczky, M. M. & Medveczky, P. G. The Lck binding domain of herpesvirus saimiri TIP-484 constitutively activates Lck and STAT3 in T cells. *J. Virol.* **73**, 1689–1694 (1999).
  70. Wehner, L. E. *et al.* Herpesvirus saimiri Tip gene causes T-cell lymphomas in transgenic mice. *DNA Cell. Biol.* **20**, 81–88 (2001).
  71. Wang, F., Kikutani, H., Tsang, S. F., Kishimoto, T. & Kieff, E. Epstein–Barr virus nuclear protein 2 transactivates a cis-acting CD23 DNA element. *J. Virol.* **65**, 4101–4106 (1991).
  72. Grossman, S. R., Johannsen, E., Tong, X., Yalamanchili, R. & Kieff, E. The Epstein–Barr virus nuclear antigen 2 transactivator is directed to response elements by the J $\kappa$  recombination signal-binding protein. *Proc. Natl Acad. Sci. USA* **91**, 7568–7572 (1994).
  73. Kaiser, C. *et al.* The proto-oncogene *c-myc* is a direct target gene of Epstein–Barr virus nuclear antigen 2. *J. Virol.* **73**, 4481–4484 (1999).
  74. Tong, X., Wang, F., Thut, C. J. & Kieff, E. The Epstein–Barr virus nuclear protein 2 acidic domain can interact with TFIIB, TAF40, and RPA70 but not with TATA-binding protein. *J. Virol.* **69**, 585–588 (1995).
  75. Wang, L., Grossman, S. R. & Kieff, E. Epstein–Barr virus nuclear protein 2 interacts with p300, CBP, and PCAF histone acetyltransferases in activation of the LMP1 promoter. *Proc. Natl Acad. Sci. USA* **97**, 430–435 (2000).
  76. Rabson, M., Gradoville, L., Heston, L. & Miller, G. Non-immortalizing P3J-HR-1 Epstein–Barr virus: a deletion mutant of its transforming parent, Jijoye. *J. Virol.* **44**, 834–844 (1982).
  77. Hammerschmidt, W. & Sugden, B. Genetic analysis of immortalizing functions of Epstein–Barr virus in human B lymphocytes. *Nature* **340**, 393–397 (1989).
  78. Cohen, J. I., Wang, F., Mannick, J. & Kieff, E. Epstein–Barr virus nuclear protein 2 is a key determinant of lymphocyte transformation. *Proc. Natl Acad. Sci. USA* **86**, 9558–9562 (1989).

79. Tomkinson, B., Robertson, E. & Kieff, E. Epstein-Barr virus nuclear proteins EBNA-3A and EBNA-3C are essential for B-lymphocyte growth transformation. *J. Virol.* **67**, 2014–2025 (1993).
80. Sample, C. & Parker, B. Biochemical characterization of Epstein-Barr virus nuclear antigen 3A and 3C proteins. *Virology* **205**, 534–539 (1994).
81. Tomkinson, B. & Kieff, E. Use of second-site homologous recombination to demonstrate that Epstein-Barr virus nuclear protein 3B is not important for lymphocyte infection or growth transformation *in vitro*. *J. Virol.* **66**, 2893–2903 (1992).
82. Le Roux, A., Kerdiles, B., Walls, D., Dedieu, J. F. & Perricaudet, M. The Epstein-Barr virus determined nuclear antigens EBNA-3A, -3B, and -3C repress EBNA-2-mediated transactivation of the viral terminal protein 1 gene promoter. *Virology* **205**, 596–602 (1994).
83. Robertson, E. S., Lin, J. & Kieff, E. The amino-terminal domains of Epstein-Barr virus nuclear proteins 3A, 3B, and 3C interact with RBPJ(κ). *J. Virol.* **70**, 3068–3074 (1996).
84. Marshall, D. & Sample, C. Epstein-Barr virus nuclear antigen 3C is a transcriptional regulator. *J. Virol.* **69**, 3624–3630 (1995).
85. Parker, G. A. *et al.* Epstein-Barr virus nuclear antigen (EBNA)3C is an immortalizing oncoprotein with similar properties to adenovirus E1A and papillomavirus E7. *Oncogene* **13**, 2541–2549 (1996).
86. Staskus, K. A. *et al.* Cellular tropism and viral interleukin-6 expression distinguish human herpesvirus 8 involvement in Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castlemans disease. *J. Virol.* **73**, 4181–4187 (1999).
87. Sadler, R. *et al.* A complex translational program generates multiple novel proteins from the latently expressed kaposin (K12) locus of Kaposi's sarcoma-associated herpesvirus. *J. Virol.* **73**, 5722–5730 (1999).
88. Kliche, S. *et al.* Signaling by human herpesvirus 8 kaposin A through direct membrane recruitment of cytohesin-1. *Mol. Cell* **7**, 833–843 (2001).
89. Muralidhar, S. *et al.* Identification of kaposin (open reading frame K12) as a human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) transforming gene. *J. Virol.* **72**, 4980–4988 (1998).
90. Muralidhar, S. *et al.* Characterization of the human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) oncogene, kaposin (ORF K12). *J. Clin. Virol.* **16**, 203–213 (2000).
91. Cesarman, E. *et al.* Kaposi's sarcoma-associated herpesvirus contains G protein-coupled receptor and cyclin D homologs which are expressed in Kaposi's sarcoma and malignant lymphoma. *J. Virol.* **70**, 8218–8223 (1996).
92. Guo, H. G. *et al.* Characterization of a chemokine receptor-related gene in human herpesvirus 8 and its expression in Kaposi's sarcoma. *Virology* **228**, 371–378 (1997).
93. Chiou, C. J. *et al.* Patterns of gene expression and a transactivation function exhibited by the vGCR (ORF74) chemokine receptor protein of Kaposi's sarcoma-associated herpesvirus. *J. Virol.* **76**, 3421–3439 (2002).
94. Arvanitakis, L., Geras-Raaka, E., Varma, A., Gershengorn, M. C. & Cesarman, E. Human herpesvirus KSHV encodes a constitutively active G-protein-coupled receptor linked to cell proliferation. *Nature* **385**, 347–350 (1997).
- Characterized the KSHV GPCR protein as a constitutively active signalling receptor protein.**
95. Gershengorn, M. C., Geras-Raaka, E., Varma, A. & Clark-Lewis, I. Chemokines activate Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor in mammalian cells in culture. *J. Clin. Invest.* **102**, 1469–1472 (1998).
96. Geras-Raaka, E., Varma, A., Clark-Lewis, I. & Gershengorn, M. C. Kaposi's sarcoma-associated herpesvirus (KSHV) chemokine vMIP-II and human SDF-1α inhibit signaling by KSHV G protein-coupled receptor. *Biochem. Biophys. Res. Commun.* **253**, 725–727 (1998).
97. Geras-Raaka, E., Varma, A., Ho, H., Clark-Lewis, I. & Gershengorn, M. C. Human interferon-γ-inducible protein 10 (IP-10) inhibits constitutive signaling of Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor. *J. Exp. Med.* **188**, 405–408 (1998).
98. Sodhi, A. *et al.* The Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor upregulates vascular endothelial growth factor expression and secretion through mitogen-activated protein kinase and p38 pathways acting on hypoxia-inducible factor 1α. *Cancer Res.* **60**, 4873–4880 (2000).
99. Montaner, S., Sodhi, A., Pece, S., Mesri, E. A. & Gutkind, J. S. The Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor promotes endothelial cell survival through the activation of Akt/protein kinase B. *Cancer Res.* **61**, 2641–2648 (2001).
100. Cannon, M., Philpott, N. J. & Cesarman, E. The Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor has broad signaling effects in primary effusion lymphoma cells. *J. Virol.* **77**, 57–67 (2003).
101. Bais, C. *et al.* Kaposi's sarcoma associated herpesvirus G protein-coupled receptor immortalizes human endothelial cells by activation of the VEGF receptor-2/ KDR. *Cancer Cell* **3**, 131–143 (2003).
102. Bais, C. *et al.* G-protein-coupled receptor of Kaposi's sarcoma-associated herpesvirus is a viral oncogene and angiogenesis activator. *Nature* **391**, 86–89 (1998).
103. Pati, S. *et al.* Activation of NF-κB by the human herpesvirus 8 chemokine receptor ORF74: evidence for a paracrine model of Kaposi's sarcoma pathogenesis. *J. Virol.* **75**, 8660–8673 (2001).
104. Montaner, S. *et al.* Endothelial infection with KSHV genes *in vivo* reveals that vGPCR initiates Kaposi's sarcomagenesis and can promote the tumorigenic potential of viral latent genes. *Cancer Cell* **3**, 23–36 (2003).
105. Yang, T. Y. *et al.* Transgenic expression of the chemokine receptor encoded by human herpesvirus 8 induces an angioproliferative disease resembling Kaposi's sarcoma. *J. Exp. Med.* **191**, 445–454 (2000).
106. Guo, H. G. *et al.* Kaposi's sarcoma-like tumors in a human herpesvirus 8 ORF74 transgenic mouse. *J. Virol.* **77**, 2631–2639 (2003).
107. Wilson, J. B., Bell, J. L. & Levine, A. J. Expression of Epstein-Barr virus nuclear antigen-1 induces B cell neoplasia in transgenic mice. *EMBO J.* **15**, 3117–3126 (1996).
108. Humme, S. *et al.* The EBV nuclear antigen 1 (EBNA1) enhances B-cell immortalization several thousandfold. *Proc. Natl Acad. Sci. USA* **100**, 10989–10994 (2003).
109. Sheu, L. F. *et al.* Enhanced malignant progression of nasopharyngeal carcinoma cells mediated by the expression of Epstein-Barr nuclear antigen 1 *in vivo*. *J. Pathol.* **180**, 243–248 (1996).
110. Tsimbouri, P., Drotar, M. E., Coy, J. L. & Wilson, J. B. *bcl-xL* and RAG genes are induced and the response to IL-2 enhanced in EmuEBNA-1 transgenic mouse lymphocytes. *Oncogene* **21**, 5182–5187 (2002).
111. Jones, R. J. *et al.* Epstein-Barr virus nuclear antigen 1 (EBNA1) induced cytotoxicity in epithelial cells is associated with EBNA1 degradation and processing. *Virology* **313**, 663–676 (2003).
112. Levitskaya, J. *et al.* Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1. *Nature* **375**, 685–688 (1995).
- Demonstrated that the Gly-Ala repeats in EBV EBNA1 inhibited CTL recognition, indicating that the EBNA1 protein can help the virus evade host immune surveillance.**
113. Levitskaya, J., Sharipo, A., Leonchiks, A., Ciechanover, A. & Masucci, M. G. Inhibition of ubiquitin/proteasome-dependent protein degradation by the Gly-Ala repeat domain of the Epstein-Barr virus nuclear antigen 1. *Proc. Natl Acad. Sci. USA* **94**, 12616–12621 (1997).
114. Dittmer, D. *et al.* A cluster of latently expressed genes in Kaposi's sarcoma-associated herpesvirus. *J. Virol.* **72**, 8309–8315 (1998).
115. Fakhari, F. D. & Dittmer, D. P. Charting latency transcripts in Kaposi's sarcoma-associated herpesvirus by whole-genome real-time quantitative reverse transcription-PCR. *J. Virol.* **76**, 6213–6223 (2002).
116. Dittmer, D. P. Transcription profile of Kaposi's sarcoma-associated herpesvirus in primary Kaposi's sarcoma lesions as determined by real-time PCR arrays. *Cancer Res.* **63**, 2010–2015 (2003).
117. Ballestar, M. E., Chatis, P. A. & Kaye, K. M. Efficient persistence of extrachromosomal KSHV DNA mediated by latency-associated nuclear antigen. *Science* **284**, 641–644 (1999).
118. Cotter, M. A., Subramanian, C. & Robertson, E. S. The Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen binds to specific sequences at the left end of the viral genome through its carboxy-terminus. *Virology* **291**, 241–259 (2001).
119. Grundhoff, A. & Ganem, D. The latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus permits replication of terminal repeat-containing plasmids. *J. Virol.* **77**, 2779–2783 (2003).
120. Garber, A. C., Hu, J. & Renne, R. LANA cooperatively binds to two sites within the terminal repeat, both sites contribute to LANA's ability to suppress transcription and facilitate DNA replication. *J. Biol. Chem.* **277**, 27401–27411 (2002).
121. Friberg, J. Jr, Kong, W., Hottiger, M. O. & Nabel, G. J. p53 inhibition by the LANA protein of KSHV protects against cell death. *Nature* **402**, 889–894 (1999).
122. Radkov, S. A., 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. *Nature Med.* **6**, 1121–1127 (2000).
- Shown that the major latent protein of KSHV, LANA, targets the Rb-E2F cellular pathway.**
123. Fujimuro, M. *et al.* A novel viral mechanism for dysregulation of β-catenin in Kaposi's sarcoma-associated herpesvirus latency. *Nature Med.* **9**, 300–306 (2003).
- Demonstrates that KSHV LANA stimulates S-phase entry and stabilizes β-catenin through a novel mechanism involving the cell cycle-dependent nuclear accumulation of its inhibitor GSK-3β.**
124. Hall, K. T. *et al.* Characterization of the herpesvirus saimiri ORF73 gene product. *J. Gen. Virol.* **81**, 2653–2658 (2000).
125. Schafer, A. *et al.* The latency-associated nuclear antigen homolog of herpesvirus saimiri inhibits lytic virus replication. *J. Virol.* **77**, 5911–5925 (2003).
126. Calderwood, M. A., Hall, K. T., Matthews, D. A. & Whitehouse, A. The herpesvirus saimiri ORF73 gene product interacts with host-cell mitotic chromosomes and self-associates via its C terminus. *J. Gen. Virol.* **85**, 147–153 (2004).
127. Verma, S. C. & Robertson, E. S. ORF73 of herpesvirus Saimiri strain C488 tethers the viral genome to metaphase chromosomes and binds to cis-acting DNA sequences in the terminal repeats. *J. Virol.* **77**, 12494–12506 (2003).
128. Hall, K. T. *et al.* The herpesvirus saimiri open reading frame 73 gene product interacts with the cellular protein p32. *J. Virol.* **76**, 11612–11622 (2002).
129. Lan, K., Kuppers, D. A., Verma, S. C. & Robertson, E. S. Kaposi's sarcoma-associated herpesvirus-encoded latency-associated nuclear antigen inhibits lytic replication by targeting Rta: a potential mechanism for virus-mediated control of latency. *J. Virol.* **78**, 6585–6594 (2004).
130. Miyazaki, I., Cheung, R. K. & Dorsch, H. M. Viral interleukin 10 is critical for the induction of B cell growth transformation by Epstein-Barr virus. *J. Exp. Med.* **178**, 439–447 (1993).
131. Zeidler, R. *et al.* Downregulation of TAP1 in B lymphocytes by cellular and Epstein-Barr virus-encoded interleukin-10. *Blood* **90**, 2390–2397 (1997).
132. de Waal Malefyt, R. *et al.* Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J. Exp. Med.* **174**, 915–924 (1991).
133. Salek-Ardakani, S. *et al.* High level expression and purification of the Epstein-Barr virus encoded cytokine viral interleukin 10: efficient removal of endotoxin. *Cytokine* **17**, 1–13 (2002).
134. Stuart, A. D., Stewart, J. P., Arrand, J. R. & Mackett, M. The Epstein-Barr virus encoded cytokine viral interleukin-10 enhances transformation of human B lymphocytes. *Oncogene* **11**, 1711–1719 (1995).
135. Bejarano, M. T. & Masucci, M. G. Interleukin-10 abrogates the inhibition of Epstein-Barr virus-induced B-cell transformation by memory T-cell responses. *Blood* **92**, 4256–4262 (1998).
136. Suzuki, T. *et al.* Viral interleukin 10 (IL-10), the human herpes virus 4 cellular IL-10 homologue, induces local energy to allogeneic and syngeneic tumors. *J. Exp. Med.* **182**, 477–486 (1995).
137. Swaminathan, S., Hesselton, R., Sullivan, J. & Kieff, E. Epstein-Barr virus recombinants with specifically mutated BCRF1 genes. *J. Virol.* **67**, 7406–7413 (1993).
138. Moore, P. S., Boshoff, C., Weiss, R. A. & Chang, Y. Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. *Science* **274**, 1739–1744 (1996).
- Describes the functions of the KSHV-encoded viral cytokines.**
139. Neipel, F. *et al.* Human herpesvirus 8 encodes a homolog of interleukin-6. *J. Virol.* **71**, 839–842 (1997).
140. Nicholas, J. *et al.* Kaposi's sarcoma-associated herpesvirus-8 encodes homologues of macrophage inflammatory protein-1 and interleukin-6. *Nature Med.* **3**, 287–292 (1997).
141. Mori, Y. *et al.* Human herpesvirus 8-encoded interleukin-6 homologue (viral IL-6) induces endogenous human IL-6 secretion. *J. Med. Virol.* **61**, 332–335 (2000).
142. Foussat, A. *et al.* Human interleukin-6 is *in vivo* an autocrine growth factor for human herpesvirus-8-infected malignant B lymphocytes. *Eur. Cytokine Netw.* **10**, 501–508 (1999).
143. Chatterjee, M., Osborne, J., Bestetti, G., Chang, Y. & Moore, P. S. Viral IL-6-induced cell proliferation and immune evasion of interferon activity. *Science* **298**, 1432–1435 (2002).
144. Molden, J., Chang, Y., You, Y., Moore, P. S. & Goldsmith, M. A. A Kaposi's sarcoma-associated herpesvirus-encoded cytokine homologue (vIL-6) activates signaling through the

- shared gp130 receptor subunit. *J. Biol. Chem.* **272**, 19625–19631 (1997).
145. Parravicini, C. *et al.* Expression of a virus-derived cytokine, KSHV vIL-6, in HIV-seronegative Castleman's disease. *Am. J. Pathol.* **151**, 1517–1522 (1997).
146. Jones, K. D. *et al.* Involvement of interleukin-10 (IL-10) and viral IL-6 in the spontaneous growth of Kaposi's sarcoma herpesvirus-associated infected primary effusion lymphoma cells. *Blood* **94**, 2871–2879 (1999).
147. Parravicini, C. *et al.* Differential viral protein expression in Kaposi's sarcoma-associated herpesvirus-infected diseases: Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. *Am. J. Pathol.* **156**, 743–749 (2000).
148. Boshoff, C. *et al.* Angiogenic and HIV-inhibitory functions of KSHV-encoded chemokines. *Science* **278**, 290–294 (1997).
149. Sozzani, S. *et al.* The viral chemokine macrophage inflammatory protein-II is a selective T<sub>H</sub>2 chemoattractant. *Blood* **92**, 4036–4039 (1998).
150. Stine, J. T. *et al.* KSHV-encoded CC chemokine vMIP-III is a CCR4 agonist, stimulates angiogenesis, and selectively chemoattracts T<sub>H</sub>2 cells. *Blood* **95**, 1151–1157 (2000).
151. Nakano, K. *et al.* Kaposi's sarcoma-associated herpesvirus (KSHV)-encoded vMIP-I and vMIP-II induce signal transduction and chemotaxis in monocytic cells. *Arch. Virol.* **148**, 871–890 (2003).
152. Weber, K. S. *et al.* Selective recruitment of T<sub>H</sub>2-type cells and evasion from a cytotoxic immune response mediated by viral macrophage inhibitory protein-II. *Eur. J. Immunol.* **31**, 2458–2466 (2001).
153. Yao, Z. *et al.* Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity* **3**, 811–821 (1995).
154. Yao, Z. *et al.* Human IL-17: a novel cytokine derived from T cells. *J. Immunol.* **155**, 5483–5486 (1995).
155. Hollyoake, M., Stuhler, A., Farrell, P., Gordon, J. & Sinclair, A. The normal cell cycle activation program is exploited during the infection of quiescent B lymphocytes by Epstein–Barr virus. *Cancer Res.* **55**, 4784–4787 (1995).
156. Nicholas, J., Cameron, K. R. & Honess, R. W. Herpesvirus saimiri encodes homologues of G protein-coupled receptors and cyclins. *Nature* **355**, 362–365 (1992).
- First paper to report the molecular piracy of cellular genes by rhadinoviruses.**
157. Ojala, P. M. *et al.* The apoptotic v-cyclin–CDK6 complex phosphorylates and inactivates Bcl-2. *Nature Cell Biol.* **2**, 819–825 (2000).
158. Verschuren, E. W., Kiefstrom, J., Evan, G. I. & Jones, N. The oncogenic potential of Kaposi's sarcoma-associated herpesvirus cyclin is exposed by p53 loss *in vitro* and *in vivo*. *Cancer Cell* **2**, 229–241 (2002).
159. Jung, J. U., Stager, M. & Desrosiers, R. C. Virus-encoded cyclin. *Mol. Cell. Biol.* **14**, 7235–7244 (1994).
160. Ensser, A. *et al.* Independence of herpesvirus-induced T cell lymphoma from viral cyclin D homologue. *J. Exp. Med.* **193**, 637–642 (2001).

**Acknowledgements**

The author would like to thank D. P. Dittmer for critical reading of the manuscript. The author is supported by grants from the American Association for Cancer Research (Gertrude B. Elion Research Award), American Heart Association and NIH/NCI.

**Competing interests statement**

The author declares that she has no competing financial interests.

 **Online links**

**DATABASES**

**The following terms in this article are linked online to:**

**Entrez:** <http://www.ncbi.nlm.nih.gov/Entrez/>  
EBV | HCMV | HHV-6 | HHV-7 | HSV-1 | HSV-2 | KSHV | MMP-9 | NF-κB | PI3K | VZV  
**SwissProt:** <http://www.ca.expasy.org/sprot/>  
Kaposin | LANA | LMP1 | LYN

**FURTHER INFORMATION**

**Blossom Damania's laboratory:** <http://www.unc.edu/~damania>

**Access to this links box is available online.**