The molecular genetics of human herpesvirus 8

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Epidemiological evidence from the early 1990s suggested that a sexually transmitted infectious agent other than the HIV virus was associated with the development of Kaposi's sarcoma in patients with HIV. A novel gamma herpesvirus was identified in Kaposi's sarcoma lesions. The molecular genetics of this virus are reviewed in this article.

Human immunodeficiency virus (HIV)-related Kaposi's sarcoma (KS) is 10 times commoner in homosexual men than in other transmission groups and is four times more frequent in HIV-positive patients who acquired their infection from blood transfusions than in haemophiliacs. These observations lead Beral et al (1990) to propose that a second infectious agent could account for the prevalence of KS in immunodeficient patients and a cohort study of gay men with AIDS suggested that frequent faeco-oral contact was a possible transmission mode for the putative infectious agent (Beral et al, 1992).

In 1994, Chang et al isolated unique DNA sequences from KS biopsies using representational difference analysis, a polymerase chain reaction (PCR)-based technique that enriches DNA fragments ('representations') present in the tumour but absent from normal tissues. The original sequences that were found were homologous to, but distinct from, capsid and tegument protein genes of the gamma herpesviruses herpesvirus saimiri and Epstein–Barr virus (EBV) (Chang et al, 1994). Complete genomic sequencing of this novel herpesvirus, initially named Kaposi's sarcoma herpesvirus (KSHV) and subsequently renamed human herpesvirus-8 (HHV8) has been completed (Russo et al, 1996). The sequence analysis confirms that HHV8 is most closely related to herpes saimiri virus which induces lymphoid malignancies in New World primates and EBV, a human oncogenic herpesvirus.

The HHV8 genome consists of a 140 kb unique coding region flanked by terminal repeat sequences. The unique region includes at least 81 open reading frames (ORFs) that potentially encode proteins. In addition to structural proteins and viral enzymes, HHV8 encodes several pirated eukaryotic cellular proteins which could maintain its latent state in B lymphocytes or in KS-associated spindle cells and contribute to tumour formation. These include homologues for a G-protein coupled receptor (GPCR), cyclin D, interleukin-6 (IL-6), Bcl-2, and interferon regulatory factor (*Figure 1*).

G-PROTEIN COUPLED RECEPTOR

The GPCR encoded by ORF 74 of HHV8 is a homologue of the human interleukin-8 receptor and is expressed in both KS lesions and in primary effusion lymphomas (Arvanitakis et al, 1997). It exhibits constitutive signalling in the absence of ligand and can activate downstream protein kinases via activation of phosphoinositide-specific phospholipase C. Furthermore, the HHV8-GPCR stimulates cell proliferation and transformation (Bais et al, 1998). This constitutive signalling can be inhibited by GPCR-specific kinases (GRKs) and GRK-5 inhibits HHV8-GPCR driven cell proliferation in rodent fibroblasts. This permanently switched on receptor has the properties of a viral oncogene and could contribute to the development of tumours in HHV8-infected cells. These observations may lead to the development of a therapeutic strategy for clinical management of HHV8 infection.

CYCLIN D

HHV8 includes a viral cyclin D gene, ORF 72, that is a homologue of the cellular cyclin D2 gene. In mammalian cells D-cyclins complex with cyclin dependent kinases (Cdk4 and Cdk6) to promote the passage of cells through the G1

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phase of the cell cycle and this activity is constrained by p16-, p21- and p27-mediated Cdk inhibition. The HHV8 v-cyclin forms an active complex with Cdk6 that promotes phosphorylation and inactivation of the retinoblastoma tumour suppressor protein. Furthermore, this v-cyclin–Cdk6 complex is resistant to Cdk inhibitors and circumvents G1 arrest (Swanton et al, 1997), suggesting that it may promote tumour formation.

INTERLEUKIN–6

The HHV8 gene K2 encodes a structural homologue of IL-6 which has conserved the region involved in receptor binding and it is notable that HHV8 is associated with diseases characterized by IL-6 dysregulation including multicentric Castleman's disease, primary effusion B cell lymphomas and myeloma.

The HHV8 vIL-6 promotes the proliferation of a human myeloma cell line that is dependent upon exogenous IL-6 for growth and survival and this effect has been shown to act via the human IL-6 receptor (Burger et al, 1998). This suggests that vIL-6 may substitute for the human cytokine and contribute to the pathogenesis of haematological malignancies. vIL-6 is expressed in both HIV-positive and HIV-seronegative Castleman's disease and is generally associated with the multicentric plasma cell variant of the disease which follows an aggressive clinical course. In contrast, vIL-6 is not expressed in KS spindle cells and its role in KS pathogenesis has yet to be elucidated.

Both HHV8 and vIL-6 have been found in the bone marrow dendritic cells of multiple myeloma patients but not in malignant plasma cells nor in bone marrow dendritic cells from normal patients (Rettig et al, 1997). Thus HHV8 may stimulate and maintain abnormal plasma cell proliferation in myeloma by ectopic production of vIL-6 in the bone marrow microenvironment, promoting survival by preventing apoptosis.

BCL–2

HHV8 sequence analysis has revealed a novel viral Bcl-2 homologue, encoded by ORF 16, designated KSBcl-2 and a similar viral Bcl-2 homologue BHRF1 is present in EBV. The Bcl-2 protein family is characterized by the ability to modulate apoptosis, and members of this family share two highly conserved domains called Bcl-2 homology 1 (BH1) and 2 (BH2) which have been shown to be critical for the death-repressor activity of Bcl-2 and Bcl-xL. KSBcl-2 overexpression blocks apoptosis and KSBcl-2 fails to heterodimerize with cellular Bax or Bak proteins which inhibit other Bcl-2 homologues. Thus KSBcl-2 may contribute to virus induced immortalization of cells.

VIRAL INTERFERON REGULATORY FACTOR

ORF K9 of HHV8 encodes a viral interferon regulatory factor (vIRF). Members of the IRF gene family encode DNA binding transcription factors that are responsible for regulating the

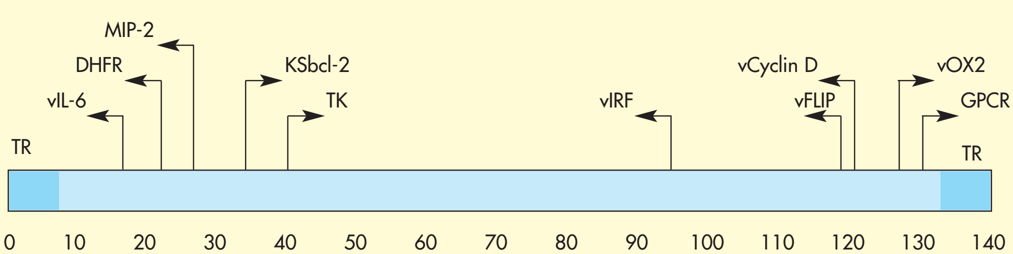

Figure 1. Human herpesvirus 8 genome and location of pirated cellular homologues. DHFR = dihydrofolate reductase; GPCR = G-protein coupled receptor; KSbcl2 = Kaposi's sarcoma b-cell lymphoma 2; MIP-2 = macrophage inhibitory protein-2; TK = thymidine kinase; TR = terminal repeat; vFLIP = viral FLICE inhibitory protein; vIL-6 = viral interleukin-6; VIRF = viral interferon regulatory factor; vOX2 = viral OX2.

transcription of interferon and interferon-induced genes. The HHV8 vIRF inhibits β-interferon-induced gene transcription, thereby blocking the host's antiviral response. NIH3T3 cells stably transfected with vIRF formed tumours when injected into nude mice, suggesting that vIRF acts as a viral oncogene. Like vIL-6, vIRF is expressed in HHV8-infected B lymphocytes where it may contribute to oncogenesis but it is not expressed in KS spindle cells.

MACROPHAGE INFLAMMATORY PROTEINS

In addition to IL-6 and IRF, HHV8 encodes two pirated CC chemokines, macrophage inflammatory proteins (MIP) I and II. vMIP–I, encoded by ORF K6, blocks HIV-1 cell entry mediated by CCR5 and inhibits replication of HIV-1 strains dependent on the CCR5 co-receptor (Moore et al, 1996). Similarly, vMIP–II, encoded by ORF K4, blocks infection by HIV-1 of a CD4 positive, CCR3-positive cell line (Boshoff et al, 1997).

It has been postulated that the inhibition of HIV entry into cells by HHV8 gene products may account for the lower rate of acquired immunodeficiency syndrome (AIDS)-related dementia observed among AIDS patients with KS as CCR3 is one of the main receptors for HIV-1 entry into microglia. However, other investigators have not found any correlation between the incidence of AIDS-related dementia and KS (Neuenburg et al, 2000). As well as blocking HIV-1 infection, both viral MIPs have been shown to be highly angiogenic in a chorioallantoic membrane assay, suggesting a possible role in the development of KS.

VIRAL FLICE INHIBITORY PROTEIN

The tumour necrosis factor receptor family cause apoptosis through a pathway culminating in the activation of the protease FLICE. Cellular inhibitors of FLICE have recently been characterized and named FLIP (FLICE-inhibitory protein). Viruses including HHV8 and molluscipoxvirus, have evolved numerous strategies to avoid host cell apoptosis including the hijacking of cellular FLIP genes. HHV8 encoded vFLIP inhibits the activation of FLICE and may prevent cellular apoptosis (Thome et al, 1997).

OTHER GENES

HHV8 encodes additional eukaryotic genes including OX2, a cell surface molecule, dihydrofolate reductase and adhesin (related to neural cell adhesion molecule). The HHV8 latent nuclear antigen 1 (LNA-1) encoded by ORF 73 resembles EBV nuclear antigens and may maintain the viral genome in an episomal form. LNA-1 is immunogenic and forms the basis for serological tests for HHV8.

HHV8 has four subtypes based on variability of the ORF K1 gene and ORF K1 has transforming properties in vitro (Lee et al, 1998) but its role in pathogenesis remains unclear. In addition, HHV8 encodes a gene homologous to the SM protein of EBV, a posttranscriptional regulator of gene expression which may be involved in KS pathogenesis (Gupta et al, 2000).

The genomic sequencing of HHV8 has uncovered a remarkable number of genes that the virus has pirated from its cellular host, including several candidate oncogenes. In vitro studies have demonstrated that primary human endothelial cells infected with HHV8 display some properties of transformation (Flore et al, 1998), but further studies are required to define its role in disease. The expression of these pirated eukaryotic genes varies between different HHV8 related malignancies. The v-cyclin expression appears to be ubiquitous while vIL-6 expression is confined to haematolymphoid cells and vGPCR expression appears to be confined to AIDS-related KS but not classical KS.

HHV8 has been demonstrated in KS lesions from patients with all forms of the disease including HIV-associated KS, endemic KS, classical KS, allograft recipients with KS and HIV-seronegative gay men with KS. In addition, two rare HIV-associated lymphoproliferative disorders have been recognized in which HHV8 has been detected. Primary effusion lymphoma is a rare high grade B-cell malignancy characterized by malignant effusions in the absence of nodal disease. Primary effusion lymphomas express an indeterminate immunophenotype with clonal immunoglobulin gene rearrangements. Castleman's disease is a multicentric angiofollicular hyperplasia that may co-exist with Kaposi's sarcoma. The multicentric variant of Castleman's disease is a polyclonal lymphoid proliferation with vascular hyperplasia causing fever, splenomegaly and lymphadenopathy. HHV8 has been detected at high frequency in both diseases.

Molecular epidemiological studies and first generation serologic assays have demonstrated that HHV8 is not ubiquitous. The virus appears more prevalent in those populations at increased risk for developing KS, such as gay men, individuals from Africa, and certain populations in Mediterranean Europe (mainly Greece and Italy), while the prevalence of HHV8 appears quite low in the general population of the USA and UK (Gao et al, 1996). Using both immunofluorescence and enzyme-linked immunosorbent assays (ELISA) for latent HHV8 antigen, 3% of blood donors from the UK, 5% of blood donors from the USA, and approximately 35% of donors from Uganda are HHV8 seropositive (Simpson et al, 1996). However, the assays used are suboptimal and even in AIDS patients with KS who probably all have HHV8, most assays are only seropositive in 80%. Part of the differences between assays may relate to the patient populations since the antibody titres recorded in endemic KS are 10 times higher than those in AIDS-related KS. A recent seroprevalence study using an ELISA assay has found that 7% of American blood donors are seropositive compared to 4% in Thailand, Malaysia and India. The same assay found up to half of American gay men and intravenous blood donors were seropositive. It is interesting that the population rates in both Thailand and Gambia are relatively high and yet both AIDS associated and classical KS are rare in these countries suggesting factors other than HHV8 infection are involved in KS pathogenesis.

The mode of transmission of HHV8 remains unclear. Initial studies suggested that the virus was present in semen; however, numerous investigators have failed to confirm this finding and the only frequent source of HHV8 from patients with KS is peripheral blood mononuclear cells. In a serial cohort study from New York that covers the period 1984–97 the annual HHV8 seroconversion rate among 245 homosexual men was 6% per annum and was higher with increasing numbers of partners. Horizontal as well as vertical transmission of HHV8 is supported by immunofluorescence assay data from twin studies in Gambia. Eight per cent of twins under 2 years of age are seropositive, suggesting vertical transmission. The seropositive rate rises with age to 65% in those aged 45–54 years of age supporting horizontal transmission of HHV8 too.

The discovery of HHV8 opens up a potential therapeutic target for the management and prevention of HHV8 associated malignancies. In vitro the most effective antiviral agent tested has been cidofovir which, in addition, inhibits vIL-6 production by KS cells in culture (Medveczky et al, 1997). Furthermore, cidofovir inhibits the development of KS xenografts in nude mice. Although it is unlikely that antiviral agents will be of significant benefit in established tumours, their role in cancer prophylaxis among HHV8-seropositive patients may prove valuable. **HM**

Conflict of interest: none.

Arvanitakis L, Geras Raaka E, Varma A, Gershengorn MC, Cesarman E (1997) Human herpesvirus KSHV encodes a constitutively active G-protein-coupled receptor linked to cell proliferation. *Nature* **385:** 347–50

Bais C, Santomassa B, Coso O et al (1998) G-protein coupled receptor of Kaposi's sarcoma associated herpesvirus is a viral oncogene and angiogenesis activator. *Nature* **391:** 86–9

Beral V, Peterman TA, Berkelman RL, Jaffe HW (1990) Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection? *Lancet* **335:** 123–8

Beral V, Bull D, Darby S et al (1992) Risk of Kaposi's sarcoma and sexual practices associated with faecal contact in homosexual or bisexual men with AIDS. *Lancet* **339:** 632–5

Boshoff C, Endo Y, Collins P et al (1997) Angiogenic and HIV-inhibitory functions of KSHV-encoded chemokines. *Science* **278:** 290–4

Burger R, Neipel F, Fleckernstein B et al (1998) Human herpesvirus 8 interleukin-6 homologue is functionally active in human myeloma cells. *Blood* **91:** 1858–63

Chang Y, Cesarman E, Pessin MS et al (1994) Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* **266:** 1865–9

Flore O, Rafii S, Ely S, O'Leary JJ, Hyjek EM, Cesarman E (1998) Transformation of primary human endothelial cells by Kaposi's sarcoma-associated herpesvirus. *Nature* **394:** 588–92

Gao SJ, Kingsley L, Li M et al (1996) KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. *Nature Med* **2:** 925–8

Gupta AK, Ruvolo V, Patterson C, Swaminathan S (2000) The human herpesvirus 8 homolog of Epstein-Barr virus SM protein (KS-SM) is a posttranscriptional activator of gene expression. *J Virol* **74:** 1038–44

Lee H, Veazy R, Williams K et al (1998) Deregulation of cell growth by the K1 gene of Kaposi's sarcoma-associated herpesvirus. *Nature Med* **4:** 435–40

Medveczky M, Horvath E, Lund T, Medveczky P (1997) In vitro antiviral drug sensitivity of the Kaposi's sarcoma-associated herpesvirus. *AIDS* **11:** 1327–32

Moore PS, Boshoff C, Weiss RA, Chang Y (1996) Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. *Science* **274:** 1739–44

Neuenburg JK, Brodt HR, Herndier BG, Schlote W (2000) Is there really a correlation between AIDS dementia and Kaposi's sarcoma? *AIDS* **14:** 94–5

Rettig M, Ma H, Vescio R et al (1997) Kaposi's sarcoma-associated herpesvirus infection of bone marrow dendritic cells from multiple myeloma patients. *Science* **276:** 1851–4

Russo JJ, Bohenzky RA, Chien MC et al (1996) Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). *Proc Natl Acad Sci USA* **93:** 14862–7

Simpson GR, Schulz TF, Whitby D et al (1996) Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. *Lancet* **348:** 1133–8

Swanton C, Mann D, Fleckenstein B, Neipel F, Peters G, Jones N (1997) Herpes viral cyclin/Cdk6 complexes evade inhibition by CDK inhibitor proteins. *Nature* **390:** 184–7

Thome M, Schneider P, Hofmann K et al (1997) Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. *Nature* **386:** 517–21

KEY POINTS

- Human herpesvirus 8 (HHV8) has been found in all forms of Kaposi's sarcoma, primary effusion lymphoma and multicentric Castleman's disease.
- HHV8 has been found in the dendritic stromal cells associated with myeloma.
- HHV8 encodes a large number of eukaryotic pirated genes which have oncogenic potential
- Serological assays for HHV8 have found that around 3% of blood donors in UK are infected.
- In vitro HHV8 is sensitive to the antiviral drug cidofovir.