Kaposi's sarcoma-associated herpesvirus: a new human tumor virus, but how?

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Kaposi's sarcoma-associated herpesvirus, or human herpesvirus 8, the most recently discovered human tumor virus, is involved in the pathogenesis of Kaposi's sarcoma, primary effusion lymphoma and some cases of multicentric Castleman's disease. It is non-pathogenic in the majority of otherwise healthy individuals but highly oncogenic in the context of HIV-1 infection and iatrogenic immune suppression, and other cofactors might exist. Several viral genes can interfere with normal cell growth and differentiation, but their precise role in oncogenesis is still under investigation.

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In the four years since its discovery[1], research on Kaposi's sarcoma-associated herpesvirus (KSHV), or human herpesvirus 8 (HHV8), has gathered pace rapidly. In spite of early reservations regarding its role as a cause of Kaposi's sarcoma (KS) and a few well-publicized detours, which have continued to find their way into some recent textbooks (e.g. Ref. 2), Hill's criteria for causality[3] have now been met for KSHV and KS (for a review, see Ref. 4). There are only two other diseases besides KS in which KSHV clearly plays a contributory role: primary effusion lymphoma (PEL; or body cavity-based lymphoma)[5] and a subset of multicentric Castleman's disease (MCD)[6], a hyperplastic B-cell lymphoproliferative disorder. Associations with various other diseases, such as multiple myeloma and sarcoid, have been described but have not withstood scrutiny (for a review, see Refs 4,7,8).

Epidemiology

How widespread is KSHV?

Despite early concerns, first- and second-generation serological assays have proved to be very useful in detecting KSHV infection. KSHV-specific antibodies were first detected using PEL cell lines[9]. The prevalence of antibodies against a latency-associated nuclear antigen [encoded by open reading frame (ORF) 73][10–12], two recombinant structural proteins in the viral capsid (vp19; encoded by ORF 65)[13,14] and envelope (gp35/37; encoded by ORF K8.1)[15,16] is relatively consistent. Despite some remaining uncertainties (see below), this suggests that this virus is uncommon in the general population of the USA, UK and central, northern and western Europe (<3–10% with regional variation), much more frequent in some Mediterranean countries such as Italy and Greece (4–35% with marked regional variation), and widespread in Africa (30–60%)[10–20,59] (for a review and more detailed references, see Refs 4,7,8). Rates of infection in other regions, such as South America and Asia, have not been studied extensively but appear to be low.

These broad geographical patterns of infection correspond roughly to KS disease rates that were present before the onset of the AIDS epidemic. Much higher seroprevalence rates were reported initially using an immunofluorescence assay on chemically induced, virus-producing PEL cell lines ('lytic' IFA)[20], but more stringent versions of the same assay[15] and an enzyme-linked immunoassay based on purified virus[21] have produced fewer positive results. The sensitivities of the latent and recombinant antigen assays are generally only 80–95%. Assays using whole virion antigens might have sensitivities approaching 100%; however, it is not yet clear to what extent this is achieved at the expense of having higher false-positivity rates (i.e. decreased specificity; for detailed discussion see Refs 4,7,8). Given recent progress, either a combination of recombinant antigens or a stepwise protocol for serological screening should provide a highly reproducible and sensitive means of detecting infection in the near future.

In spite of some remaining uncertainties, the fact that KSHV is more common in some countries and among some risk groups than in others is reproducible with different assays. The higher prevalence of KSHV in Italy compared with that in the UK, the USA and France is supported by several PCR-based studies on peripheral blood mononuclear cells, lymphoid tissue and semen samples (reviewed in Refs 4,7,8). Although KSHV infection (like other

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herpesviruses) is presumed to be life-long, PCR testing of semen and peripheral blood is far less sensitive than serological methods and underestimates prevalence by a factor of >2–5 (Refs 4,7,8,22). Thus, studies of this kind are not suitable to estimate prevalence. For most immunocompetent individuals infection remains subclinical. In KSHV-infected individuals who become immunosuppressed during AIDS or transplantation, evidence suggests a very high rate of disease expression, perhaps >25% (C. Parravicini and D. Farge, unpublished).

How is KSHV transmitted?

Sexual transmission might play an important role in transmission of KSHV among homosexual men of Western countries, in whom this virus is more prevalent than in other individuals at risk for HIV infection, including intravenous drug users, patients with hemophilia and women, and the general population of the same country. Thus, 20–40% of homosexual men in Denmark, the UK, USA and Holland have antibodies against defined KSHV antigens. Seropositivity, and sometimes seroconversion, is associated with increasing numbers of sexual partners, a history of other sexually transmitted diseases (in some studies), receptive anal intercourse and, in one study, oral–genital contact[23–26]. One study[24] of Danish homosexual men found evidence that KSHV entered the Danish homosexual community through contact with homosexual men from the USA. This is consistent with epidemiological studies on KS published prior to the discovery of KSHV, which suggest that the 'KS agent' might have been exported independently to other countries from USA epicenters of the AIDS epidemic[27], and points to an unrecognized KSHV epidemic among homosexual men prior to the introduction of HIV into these communities. Declining KS incidence and KSHV infection rates among homosexual men since the early 1980s have been attributed to the implementation of safe-sex guidelines[24]. KSHV DNA is detected only rarely by nested PCR in the semen of KSHV-infected asymptomatic individuals (reviewed in Ref. 22). Although this could theoretically account for sexual transmission of KSHV, it is still uncertain whether the low amount of KSHV in semen is sufficient for sexual transmission or whether the sexual risk factors mentioned above only reflect other forms of intimate contact involving an exchange of other body fluids. Oral–genital contact as a risk factor for KSHV infection[26] might result from the documented presence of KSHV in saliva (reviewed in Refs 4,7,8).

At present, there is only limited evidence for sexual transmission of KSHV among heterosexuals. In the USA, Haiti and Italy, KSHV seroprevalence has been found to be higher in HIV-1-infected women than in HIV-1-uninfected women, but this association was not significant in any of these studies (e.g. see Ref. 28). However, among women in Cameroon, KSHV infection is significantly associated with HIV-1 infection and commercial sex work[29]. In one study of Italian adults[59], but not in another study[17], KSHV antibody prevalence was slightly higher in men than in women. Thus, it is not clear why classic KS occurs more frequently in men than in women.

In KSHV-endemic countries, transmission in childhood seems to account for much of the spread of KSHV. In Uganda, KSHV seroprevalence reaches adult levels well before puberty and might be associated with hepatitis B infection, suggesting that living conditions that predispose to the spread of hepatitis B virus in African children also promote the dissemination of KSHV (Ref. 18). Continuing transmission in adulthood and similar infection rates among men and women[19,30] are consistent with a nonsexual route of infection in these countries. By contrast, KSHV infection among children in the USA and UK is rare[13,31].

KSHV infection can occur during organ transplantation[32–34], but, in KSHV-endemic countries, most transplant KS patients appear to have been infected prior to transplantation[33,34]. Although concerns about transmission through blood transfusions have been raised, no clear evidence for this route of transmission has yet been described.

KSHV infection and the development of KS

KSHV can be found in >90% of HIV-associated and HIV-free forms of KS (reviewed in Refs 4,7,8). Among HIV-infected individuals, the reported incubation periods (i.e. the time from KSHV infection to the appearance of KS lesions) vary between studies[10,23,25] but might be influenced by the degree of immunosuppression in these patients. In one study of AIDS–KS, faster progression to KS occurred among men infected first with HIV-1 and then with KSHV than among men infected with KSHV and secondarily with HIV-1 (Ref. 25). One case report demonstrates that primary KSHV infection in an HIV-positive man can result in the development of KS and Castleman's-like disease in a matter of weeks[35]. Apart from KSHV infection and immunosuppression, other factors might be involved in determining progression to KS disease. One intriguing study demonstrates that HIV-1 infection is more likely than HIV-2 infection to result in KS disease among KSHV-infected patients in The Gambia[36].

KSHV, the virus

This strong association between KSHV and KS leads to questions about how KSHV induces the proliferation and atypical differentiation of endothelial cells, the likely precursors of KS spindle cells. An alluring feature of KSHV is the presence of several viral genes that are homologous to those encoding cellular proteins involved in growth control and differentiation (for a review, see Refs 4,7,37) and are thought to have been 'pirated' at some point during its evolution. Such 'molecular piracy' is a common feature of many rhadinoviruses (γ_2-herpesviruses), of which KSHV is a member, but also occurs in other herpesviruses and poxviruses[37,38]; this might represent a viral response to antiviral defense mechanisms. Individual rhadinoviruses differ with respect to the cellular genes (see Table 1) that they have 'pirated' (cytokines,

Table 1. Functional properties of KSHV genes that might play a role in pathogenesis[a]

KSHV protein	KSHV gene	Functional properties	Refs
v-cyc	*ORF 72*	Interacts with cdk6 to inactivate RB by phosphorylation, thereby releasing E2F from RB, leading to activation of E2F-dependent promoters; resistant to inhibition by p16, p21 and p27 and, therefore, mediates G1/S transition in quiescent fibroblasts; with cdk6, phosphorylates p27, thus mimicking the effect of cyclin–cdk2 complexes on p27 levels and G2/M transition; mRNA expressed in KS spindle cells and PEL cells *in vivo*	61–63; reviewed in 4,7,37,38
v-FLIP	*ORF K13* (*ORF 71*)	Homolog of cellular apoptosis inhibitor; a similar protein in HVS inhibits apoptosis; translated from bicistronic mRNA that also encodes v-cyc and is expressed in tumor cells *in vivo*	Reviewed in 4,7,37,38
LNA	*ORF 73*	Function not known, but might be an immediate-early transactivator; protein expressed in KS and PEL tumor cells *in vivo*	10,11; reviewed in 4,7,37,38
K1 protein	*ORF K1*	No cellular homolog; membrane protein, transforms rodent fibroblasts and primary T cells; recruits Syk, vav, PI3 kinase to cell membrane; induces Ca^{2+} influx; probably not a latent protein; no evidence to date for expression in tumor cells *in vivo*	50,51; reviewed in 4,7,37,38
v-GCR	*ORF 74*	Chemokine receptor homolog; transforms rodent fibroblasts and induces expression of angiogenic further growth factors, including VEGF; activation of cellular genes might involve downstream signaling through heterotrimeric G proteins, JNK/SAPK, p38/HOG; probably not a latent protein; no evidence to date for expression in tumor cells *in vivo*	54; reviewed in 4,7,37,38
v-IRF-1	*ORF K9*	Inhibits interferon-induced signaling, IRF-1-mediated transcription; transforms rodent fibroblasts; protein is not expressed in the majority of KS or PEL tumor cells *in vivo*	52,53
'Kaposin' (T0.7)	*ORF K12*	Small hydrophobic membrane protein whose mRNA is strongly expressed in tumor cells *in vivo*; thought to transform rodent fibroblasts; other differentially transcribed/translated mRNAs are expressed from this gene, whose function is still unknown	44,47,48
v-IL-6	*ORF K2*	Stimulates growth of plasma cells; binds to the subunit (gp130) of the IL-6 receptor; protein expressed in PEL tumors and MCD *in vivo*	Reviewed in 4,7
vbcl-2	*ORF 16*	Inhibits bax-mediated apoptosis	Reviewed in 4,7
vMIP-I	*ORF K6*	Induces angiogenesis	58
vMIP-II	*ORF K4*	Binds to CCR3; induces eosinophil chemotaxis and angiogenesis; induces Th2 chemotaxis	56,58
vMIP-III	*ORF K4.1*	Induces Th2 chemotaxis; protein expressed in KS lesions (by western blot)	41[b]

[a]Abbreviations: CCR, chemokine receptor; cdk, cyclin-dependent kinase; HVS, herpesvirus saimiri; IRF-1, interferon regulatory factor 1; JNK, Jun amino-terminal kinase; KSHV, Kaposi's sarcoma-associated herpesvirus; LNA, latent nuclear antigen; MCD, multicentric Castleman's disease; MIP, macrophage inflammatory protein; ORF, open reading frame; PEL, primary effusion lymphoma; RB, retinoblastoma protein; SAPK, stress-activated protein kinase; v-cyc, viral cyclin homolog; VEGF, vascular endothelium growth factor; v-FLIP, homolog of FLICE-dependent apoptosis inhibitor; v-GCR, viral homolog of G protein-coupled receptor, v-IL-6, viral homolog of interleukin 6; v-IRF-1, viral homolog of interferon regulatory factor 1.
[b]J. Stine, unpublished.

antiapoptotic factors, G-protein-coupled receptors and cell cycle regulatory proteins), and some genes might have been 'highjacked' at very different points during the evolution of this group of viruses.

Herpesvirus saimiri (HVS) of squirrel monkeys, the prototype of rhadinoviruses, was, until recently, regarded as being most closely related to KSHV (Ref. 9). However, it has recently become apparent that more closely related rhadinoviruses exist among Old World monkeys, including several macaque species and African Green monkeys[39,40] [Greensill, J. *et al.* (1998) First International Workshop on KSHV and Related Viruses, Santa Cruz, CA, USA]. Primate rhadinoviruses can be grouped broadly into two clusters using sequence analysis and biological properties. One group, including HVS and rhesus rhadinovirus[39],

appears to be readily culturable in standard cell lines, such as owl monkey kidney cells. The other group, including KSHV and two viruses detected by molecular techniques in retroperitoneal fibromatosis lesions from *Macacca nemestrina* and *Macacca mulatta*[40], do not grow readily in tissue culture.

Evolution of KSHV

Like that of other rhadinoviruses, the 165-kb KSHV genome consists of a single long unique region (LUR), containing all of the coding sequences, which is flanked on either end by direct terminal repeat sequences[41]. Although most of the LUR is highly conserved, marked sequence variability occurs in the regions of the LUR adjoining the terminal repeats. At the left end, a viral gene (*ORF K1*), encoding a membrane glycoprotein expressed during the lytic replicative cycle, has evolved into three or four groups (A, B, C and possibly D), the protein sequences of which may vary by up to 40% (Ref. 60). Evolution of *ORF K1* is driven by an unknown selective pressure, as shown by the high rate of non-synonymous to synonymous substitutions[60,64]. Group B isolates predominate in Africa, whereas group A and C isolates occur in all parts of Europe and the USA (Refs 60,64). The right end of the viral genome is also variable, and two sequence variants exist that have been speculated to be the result of a recombination event with a closely related virus[42].

Is KSHV a transforming virus?

Although epidemiological studies provide strong evidence for a role of KSHV in causing KS, the question of whether or not KSHV is a tumor virus capable of directly transforming infected cells has been a focus for debate. The answer is in the affirmative, at least for PEL, as PEL tumors can demonstrate a monoclonal virus terminal repeat pattern[41] and are fully immortalized and clonal tumors. However, for KS, the issue is more difficult to approach. *In situ* PCR, *in situ* hybridization and immunohistochemistry demonstrate that nearly all tumor cells in KS tumors do appear to be infected, suggesting that viral gene products are directly causing cell proliferation[7,11,43,44]. This does not mean that the tumor itself is, or has to be, monoclonal (cf. Epstein–Barr virus-related lymphoproliferative disorders); indeed, KS lesions might represent a polyclonal or hyperplastic outgrowth. Tumor monoclonality studies have been contradictory, possibly partly owing to the mixed neoplastic and reactive components in a typical KS lesion[4,7].

As viral tumorigenesis is thought to be a feature of latent, not lytic, viral replication, a major research priority has been the identification of latency-expressed genes that might contribute to cell transformation. It is becoming increasingly clear that viral transcriptional programs are tissue dependent. Therefore, the knowledge gained from examination of PEL cells in tissue culture might not be directly applicable to KS and MCD lesions. Indeed, preliminary evidence suggests that some genes expressed in PEL tissue culture are not expressed as proteins in PEL tumors *in situ*. The vast majority of KS tumor cells has a highly restricted pattern of gene expression, which includes a cluster of latent (type I) genes that are expressed from alternatively applied polycistronic transcripts on the right end of the genome[45,46]. These genes encode LNA (*ORF 73*), the major latent antigen, as well as v-cyc (*ORF 72*) and v-FLIP (*ORF K13*), which have the capacity to abrogate the G1 checkpoint by retinoblastoma protein phosphorylation downregulation of the cdk inhibitor p27 and to prevent apoptosis, respectively[4,7,61–63].

An interesting aspect of this gene cluster is that it is under cell cycle regulation[46]. This might allow the viral cyclin, for example, to maintain cell cycling in place of cellular cyclins that are downregulated in response to viral infection. In addition, KS tumor cells express several highly abundant small mRNAs extending into ORF K12 with alternative transcriptional and translational patterns, depending on the tissue source[44,47] [Sadler, R. *et al.* (1998) First International Workshop on KSHV and Related Viruses]. One of these mRNAs encodes a small hydrophobic protein that might have transforming capacity[48]. In PEL cells, many of the remaining cell-homologous genes are expressed at low levels during exponential cell growth but can be induced into high-level expression during the transition to lytic replication (type II transcription)[49]. The remainder of the viral genes, including those encoding most structural protein genes and those encoding homologs of DNA-synthesis enzymes, generally have an expression pattern consistent with late lytic replication (type III transcription)[49]. There are three type II/III genes for which there is experimental evidence of transforming properties but that are probably not expressed in latently (persistently) infected KS spindle cells. These are *ORF K1* (Ref. 50), which can activate immunoreceptor-tyrosine kinase

Questions for future research

- Are sera that react only in an immunofluorescence assay on productively ('lytically') infected cells, but not with any of the known immunogenic proteins [latent nuclear antigen (encoded by *ORF 73*), vp19 (*ORF 65*), gp35–37 (*ORF K8.1*)], genuinely reactive with Kaposi's sarcoma-associated herpesvirus (KSHV) proteins, and how do we corroborate their reactivity?
- How is KSHV transmitted? What are the practices that lead to its transmission among homosexual men? What are the routes of non-sexual transmission?
- Are there other cofactors that explain why African endemic KS is confined mainly to East/Central Africa, and what are they?
- Do viral homologs of chemokines and interleukin 6 contribute to KSHV-related neoplasia? Does the role of KSHV in the development of KS or multicentric Castleman's disease lesions therefore represent a 'prototype' for indirect mechanisms of neoplasia?
- How can the viral homologs of cellular growth factors, growth factor receptors, anti-apoptotic proteins and D-type cyclin be exploited to understand the evolution of their roles in controlling cellular growth and differentiation?

signaling[51]; *v-IRF-1* (*ORF K9*), which inhibits interferon signaling[52,53] and binds the transcriptional coadaptor p300 (P.S. Moore and Y. Chang, unpublished) and *v-GCR* (*ORF 74*), which acts as a constitutively active CXC chemokine receptor[54]. Whether these contribute to KS pathogenesis is, therefore, still under investigation.

It is possible that the unusual nature of KS tumors might involve novel mechanisms of pathogenesis that go beyond those encountered in other tumor viruses. A recent report suggests that KSHV might immortalize primary endothelial cells in culture, leading to the growth of KSHV-infected cells on soft agar[55]. Interestingly, KSHV-infected and -uninfected endothelial cells in these cultures show increased vascular endothelial growth factor (VEGF)-dependent proliferation, suggesting that a paracrine process could be involved in addition to direct transformation. KSHV encodes several cytokines, including an interleukin 6 homolog (v-IL-6) and several homologs to macrophage inflammatory proteins (vMIPs)[56]. v-IL-6 is likely to be the primary component responsible for B-cell proliferation in Castleman's disease[57], whereas vMIP-I and vMIP-II have angiogenic properties[58]. The GCR can also induce secretion of VEGF, the major angioproliferative cytokine[54]. Caution is required, as the expression patterns for these proteins in KS lesions are unclear, but these studies point to the intriguing possibility that KS tumorigenesis might, at least in part, be indirectly mediated by viral or cellular cytokine dysregulation.

Conclusion

KSHV is a new human tumor virus that is prevalent in Africa and parts of southern Europe but is less common in other countries. Analogous to its distant relative, Epstein–Barr virus, it appears to cause disease only rarely in the immunocompetent host but is highly oncogenic when combined with immunosuppression and HIV-1 infection. *In vitro* studies underline its potential to transform endothelial cells and have highlighted at least four potential viral oncogenes, whose precise contribution to KS pathogenesis and cell transformation is under active investigation.

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