Kaposi's Sarcoma-Associated Herpesvirus: General Features☆

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History

Kaposi's sarcoma (KS) was first described by Moritz Kaposi (born Moriz Kohn in Kaposvar, Austro-Hungary) in 1872, as a rapidly fatal, 'idiopathic, multiple pigmented sarcoma' of the skin. Although initially associated with men of Eastern European, Ashkenazi Jewish and Mediterranean ancestry, by the 1950s KS was recognized to be the third most common malignancy in sub-Saharan Africa. The unusual geographic distribution of this cancer led to speculation about an infectious agent that intensified with the onset of the acquired immune deficiency syndrome (AIDS) epidemic in the early 1980s. AIDS patients were 100 000-fold more likely to develop KS than the general population and the tumor took on a more aggressive and frequently lethal course in AIDS patients.

Two epidemiologists, Harold Jaffe and Valerie Beral, observed differences in KS incidence within various human immunodeficiency virus (HIV) transmission groups and concluded that KS is caused by 'a transmissible agent' other than the HIV and that the putative agent is uncommon in the general population but sexually transmitted efficiently among gay men. These predictions were later found to be strikingly accurate. Over 20 possible culprits were described as 'the KS agent'. As early as 1972, Giraldo and colleagues found herpes-virus like particles in KS tissues by electron microscopy and suggested that cytomegalovirus which is ubiquitously found in human population, might cause KS. These findings were discounted after further study, but in 1984 Walter and colleagues succeeded in directly observing herpesvirus particles in KS tumor specimens, thus reviving the possibility that KS is a herpesvirus disease.

KSHV was identified in KS tumors in 1993, and published in 1994, when Yuan Chang and Patrick Moore used representational difference analysis in a directed search that isolated two small DNA fragments from a KS lesion similar to, but distinct from, known herpesvirus sequences. Collaborating with Ethel Cesarman, they were then able to show that this putative new virus was present in virtually all KS tumors, was more likely to be found at the tumor site than distal tissues, and was not present in control tissues from patients without AIDS. The virus was given the common name Kaposi's sarcoma-associated herpesvirus (KSHV) and recognized to be the eighth human herpesvirus (HHV-8). The virus was also found in a subset of AIDS-associated lymphomas known at the time as body cavity-based lymphomas, and later renamed primary effusion lymphomas (PEL). Unlike KS tumors PEL maintain the virus at far higher copy number. Cell lines were quickly identified by this group allowing them to first establish the virus in culture and to develop first-generation serologic assays for antibodies against the virus. A second neoplastic disorder called multicentric Castleman's disease (MCD) was subsequently recognized to be frequently infected with KSHV by Soulier and colleagues.

Once the virus was identified, its characterization proceeded rapidly. Studies demonstrated that all forms of KS, both HIV-positive and HIV-negative, are uniformly infected with KSHV. Extensive HIV/AIDS cohort databanks were used for serologic test development and showed that KSHV infection preceded onset of disease. These early studies also revealed that KSHV infection is uncommon in the general population of the USA and Europe – despite technical confusion resulting from highly cross-reactive assays – and patterns of infection mirror KS tumor rates among high-risk populations, following closely the Beral–Jaffe predictions

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for the KS agent. Parravincini and colleagues showed that KS arising among transplant patients could either be due to reactivation of preexisting infection or to *de novo* infection from the organ allograft, helping to explain the association of this tumor with transplantation.

Virologic characterization was equally rapid. In 1996, Ganem's group isolated a KSHV-infected PEL cell line free of EBV infection, and performed the first studies on viral replication. While KSHV had been previously cultured *in vitro* in EBV-coinfected cell lines, this was a major breakthrough allowing unambiguous characterization of the virus. That same year, Chang and Moore finished sequencing the 165 kbp viral genome, almost exactly 2 years after reports of its initial discovery. These studies revealed that the virus makes extensive use of molecular piracy, encoding homologs to multiple known cellular oncogenes. Shortly thereafter, research groups identified KSHV inhibitory proteins targeting p53 and retinoblastoma protein, interferon regulatory proteins, and KSHV paracrine-signaling molecules regulating cell growth control, providing key molecular clues on how this virus might initiate tumorigenesis.

Shortly after its initial description, KSHV was widely recognized to be the infectious cofactor causing KS and related diseases. KSHV remains responsible for enormous, unappreciated morbidity and mortality especially in sub-Saharan African countries. In contrast, the introduction of highly effective retroviral therapy to developed countries in the mid-1990s has reduced KS incidence among AIDS patients by nearly 90% – a remarkable public health success story.

Taxonomy, Classification, and Evolution

KSHV is a gammaherpesvirus (subfamily *Gammaherpesvirinae*) belonging to the genus *Rhadinovirus*, which is distinct from the genus *Lymphocryptovirus* containing EBV (Figure 1). The designation '*rhadino*' comes from the Greek word for 'slender' or 'fragile' referring to the tendency of the virus genome to break apart into two fractions of highly different density. While rhadinoviruses are distributed among both primates and non-primates (e.g., mice, cattle, and horses), EBV-like viruses are found exclusively among primates.

The genus *Rhadinovirus* contains three lineages of primate viruses. One lineage is found among New World monkeys (e.g., herpesvirus saimiri, HVS) and two parallel, but distinct lineages (including KSHV), infect Old World primates. HVS is a T-lymphotropic herpesvirus of squirrel monkeys that is potently oncogenic to other New World primates and has been used to study T-cell immortalization and transformation. After the discovery of KSHV, a group led by Tim Rose reported identification of a new KSHV-like virus in rhesus macaques, providing the first indication that the KSHV-like lineage is distributed among Old World primates as well as humans. During ELISA screenings for rhesus monkey antibodies cross-reacting with HVS, Ronald Desrosiers discovered the third rhadinovirus lineage in rhesus macaques (and hence commonly called rhesus rhadinovirus, RRV). Members of this lineage were quickly detected in other Old World primate hosts. Humans are the only great apes lacking a representative RRV-like virus. This theoretical human virus has not been detected thus far despite targeted searches. The non-human primate viruses serve as important model systems to investigate rhadinovirus pathogenesis and may be useful vaccine models for KSHV.

Molecular epidemiology studies based on variations of the K1 gene, located at the left-most coding region of the genome, along with variations at the right-hand end of the genome reveal KSHV to be comprised of four unique viral clades designated A, B, C, and D. These clades have been shown by Gary Hayward to largely match patterns of human migration from Africa. Additionally, there exist several conserved alleles of the right-hand end of the genome (called M, N, O, and P) found across clades suggesting that KSHV strains can arise by recombination.

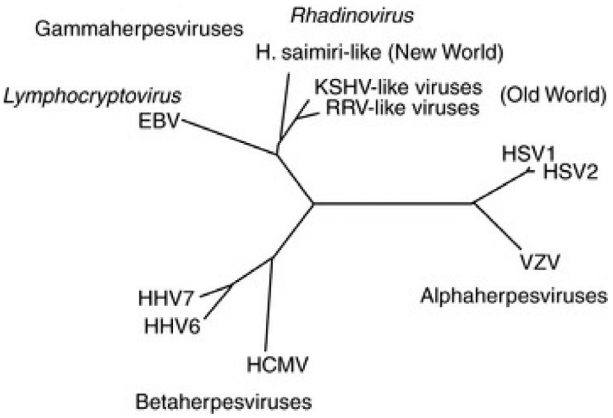

Figure 1 Relationship of KSHV to other herpesviruses. Phylogenetic trees are based on the comparison of aligned amino acid sequences among herpesviruses for the major capsid protein. KSHV belongs to a lineage of rhadinoviruses infecting Old World primates. A second rhadinovirus lineage, exemplified by rhesus rhadinovirus (RRV), has been discovered infecting Old World primates. No human infections with an RRV-like virus have been described.

Geographic Distribution and Prevalence

Unique among human herpesviruses, KSHV is not a ubiquitous human infection. KSHV infection is hyperendemic throughout sub-Saharan Africa with estimated adult infection rates of 30–60%. Other isolated populations, including indigenous Amazonian tribes (Amerindians) and some ethnic groups in China, may also have infection rates at this level or higher. The prevalence of infection shows a geographic gradient in Mediterranean basin countries such that general adult infection rates in Sicily average about 10% and continue to decline at more northern latitudes in Italy. By contrast in the USA and Northern Europe, approximately 2% of blood donors are KSHV infected. While general infection rates are low in the USA and Europe, gay men are recognized to have a rate of infection 10–20 times higher than heterosexuals.

KSHV encodes multiple antigenic proteins and thus infection with KSHV generates a strong antibody response that can be measured by indirect immunofluorescence assays, ELISA, and Western blotting. Humoral responses to these viral proteins are variable. For instance, the latency-associated nuclear antigen (LANA)1 commonly elicits antibody titers exceeding 1:50 000 in LANA1-positive patients but only ~80% of known KSHV-positive patients have positive LANA tests. LANA1 immunoreactivity is highly specific for KSHV infection but its low sensitivity precludes its routine use as a single antigen in screening tests. For this reason, attempts to increase test sensitivity using combinations to antigens or new formats for antibody testing, such as quantitative luciferase immunoprecipitation systems (LIPS), have been examined. Complicating the serologic detection of KSHV is the fact that much of this work is based on patients with end-stage AIDS-KS. These patients routinely lose antibody reactivity due to loss of CD4+ helper activity.

Transmission

In the absence of underlying immunosuppression, KSHV infection appears to be asymptomatic, or perhaps associated with transient fever and rash. KSHV is recognized to have several patterns of transmission, and modes of transmission differ dramatically between developed and developing countries. Encapsidated virus is secreted from the oral mucosa through saliva. In developed countries, there is a strong sexual component to transmission among gay and bisexual men but the precise sexual behavior responsible for transmission is unknown. Given likely oropharyngeal shedding of the virus, possibilities include deep kissing and use of saliva as a sexual lubricant. In contrast, heterosexual transmission is apparently rare.

In developing countries, and possibly endemic regions of Europe and the Middle East, casual transmission is common. Again precise mechanisms of transmission are unknown, but KSHV transmission is much more efficient in sub-Saharan Africa where, unlike Northern Europe and the USA, significant rates of infection occur among children and adolescents. While intrauterine transmission and transmission through breast milk is infrequent, maternal–child transmission does occur during infancy and early childhood. Mothers with high levels of KSHV DNA in the saliva are at risk to have KSHV-infected children.

Of increasing concern, KSHV is also transmitted both through organ allografts and through blood transfusion. KS arising during transplantation is an important clinical problem, with rates among all transplant patients between 0.2% and 6% depending on the geographic locale and background endemicity for viral infection. KS in this setting has a high mortality and morbidity since primary treatment involves removing immunosuppression. The bulk of KS among transplant recipients derives from reactivation of preexisting, quiescent infection that subsequently flares during immunosuppression, but transmission from donor allografts has also been documented. Interestingly, one study demonstrated that KS tumor cells rather than virus itself can be transmitted during transplantation.

Blood transfusion has in the past been considered a minor component of KSHV transmission. Several studies have shown a relationship between non-AIDS-KS and past transfusion history, but this relationship has been overshadowed by the overwhelming risk of sexual transmission among HIV/AIDS patients. Transfusion transmission has recently been documented to occur in sub-Saharan African countries in which whole blood is used for transfusion in malaria patients.

Host Range and Virus Propagation

KSHV can be grown in the laboratory using PEL-derived B-cell lines and can be activated into lytic virus production by treatment with chemicals including protein kinase C activators or histone deacetylase inhibitors. The virus cannot be maintained in cultured cells from KS tumor explants. KSHV can, however, be readily transmitted to a range of human and non-human cell lines in culture, The virus is commonly transmitted to endothelial cell lines, which undergo transformation into a spindle growth pattern resembling KS. Receptors for virus binding and entry include extracellular heparan, integrin $\alpha 3\beta 1$, the cystine transporter xCT, and DC-SIGN. It is not yet clear which of these receptors act in concert or independently of each other to allow different steps of binding, internalization, and entry.

Highly efficient KSHV infection and transformation model using rat embryonic mesenchymal precursor cells has been reported in which latent and inducible lytic infection could be established. Mouse model infection systems have also been developed. KSHV BAC36 transfected mouse bone marrow, endothelial linage cells develop KS-like tumors in nude mice that may be useful for understanding KS pathobiology and drug-testing analyses. A human bone marrow, liver, thymus xenograft mouse model has also

been described that efficiently maintains virus replication. Among primates, KSHV transmission to common marmosets has been reported, in which KS-like skin lesions express latent and lytic viral gene products.

In addition to whole virus infection in animal models, single gene expression studies have been extensively explored in animal models as well. The K1 protein, for example, has been placed into a HVS backbone and shown to cause lymphatic tumors in rhesus macaques. KSHV genes, including those encoding viral interleukin 6 (vIL6), viral interferon regulatory factor 1 (vIRF1), and kaposin, transform rodent cell lines and have been used in transgenic models. The expression of the viral G protein-coupled receptor (vGPCR) in transgenic mice results in paracrine development of endothelial cell tumors that closely resemble human KS tumors.

Virus Genetics and Molecular Biology

KSHV genome consists of approximately 165 kbp of linear double-stranded DNA. During lytic replication, KSHV genome is packaged as a linear DNA molecule in capsids. Sequencing analysis from established PEL cell lines (BC1 and BCBL1) shows that KSHV retains characteristic features of rhadinoviruses including a long unique region (LUR) with low G + C content (L-DNA) that is flanked by high G + C content direct terminal repeats on both ends (TRs or H-DNA). The non-coding TRs are 801 bp in length each, with 20 or more units in a typical virus. During latent virus replication, the KSHV genome is maintained in the cell nucleus as a naked circular molecule (episome) without free ends. The LUR encodes over 90 open reading frames (ORF) and at least 25 miRNAs. Among these genes, 68 ORFs are conserved between KSHV and HVS and are numbered from left to right according to their corresponding HVS homologue. They are clustered in seven gene blocks and largely encode for structural proteins as well as proteins involved in lytic replication and packaging. KSHV genes without known homology to HVS genes are designated K1 through K15 (**Figure 2(a)**).

During latency the KSHV genome is essentially quiescent with only a few genes constitutively expressed including LANA1, viral cyclin D (vCYC), viral FLICE-inhibitory protein (vFLIP), kaposins and viral miRNAs through alternative splicing of the latency transcript located at the right end of the LUR (**Figure 2(a)**). These transcripts regulate cell growth and proliferation, angiogenic and inflammatory signals, evasion from apoptosis, autophagy and contribute to cell transformation. Virus-encoded miRNAs play a critical role in the establishment and/or maintenance of latency, and also mediate cellular transformation and tumorigenesis by altering diverse pathways. LANA1 functions as a bridge between the viral episome and host cell chromosomes during latency, allowing equal segregation of virus to daughter cells. During latent replication, in which the virus is in a circular state, the origin of DNA replication is located within the TR region.

KSHV, similar to other herpesviruses, has a highly choreographed cascade of gene expression during lytic replication. Lytic replication is initiated by a single transactivator protein, RTA, expressed from ORF50. This leads to sequential early, delayed-early, and late gene expression. Ultimately, the viral polymerase is expressed and linear KSHV chromosomes are replicated through a rolling circle mechanism. Among early transcripts, polyadenylated nuclear RNA (PAN) RNA is the most abundant and was shown to bind and relocalize poly(A) binding protein to the nucleus and may play a pivotal role in new virus formation by driving late gene expression.

Herpesvirus genes are commonly divided into 'latent' and 'lytic' classes based on whether or not they are activated during the lytic replication cycle. However, in KSHV, a large group of nonstructural protein genes, are expressed at low levels during viral latency but are upregulated during lytic replication (designated as type II genes). Highly expressed PAN and kaposin RNAs, which originally were assumed to distinguish lytic and latent viral infections, are both type II transcripts that are expressed at low levels during latency, but are induced to high expression during lytic replication. Notch signaling, which targets the transcription factor RBP-Jk, acts in concert with RTA to initiate lytic replication, and can activate expression of these genes without late gene expression or virion production. Increased notch signaling is documented in PEL cells and appears to be responsible for low-level expression of type II genes even during viral latency. Further, tissue-specific expression alters gene expression patterns.

KSHV similar to other viruses expands its coding capacity by diverse mechanisms such as alternative splicing, polyadenylation, viral mRNA editing and alternative translation initiation. Genome-wide surveys with combined transcriptome and proteome analysis revealed new genomic features such as small upstream ORFs and ribosome occupancy of viral non-coding RNAs.

Molecular Piracy by KSHV

Rhadinoviruses show the most extensive piracy of host genes among the herpesviruses. KSHV genes that have been directly acquired from the host genome include those encoding proteins involved in DNA synthesis (thymidine kinase, dihydrofolate reductase, thymidylate synthase, ribonucleotide reductase subunits, and formyglycinamide ribotide amidotransferase (FGARAT)), deoxyuridine metabolism (dUTPase), paracrine signaling (viral complement control protein, vIL6, viral chemokine ligands 1, 2 and 3 (vCCL1, vCCL2, and vCCL3)) and vGPCR, interferon signaling (vIRF1, vIRF2, and LANA2), major histocompatibility class (MHC) regulation (modulator of immune recognition 1 and 2 (MIR1 and MIR2)), and cell cycle (vCYC) and apoptosis control (vBCL-2, vFLIP, and viral inhibitor of apoptosis (vIAP)). These proteins frequently fall into the type II expression pattern described above (**Figure 2(a)**).

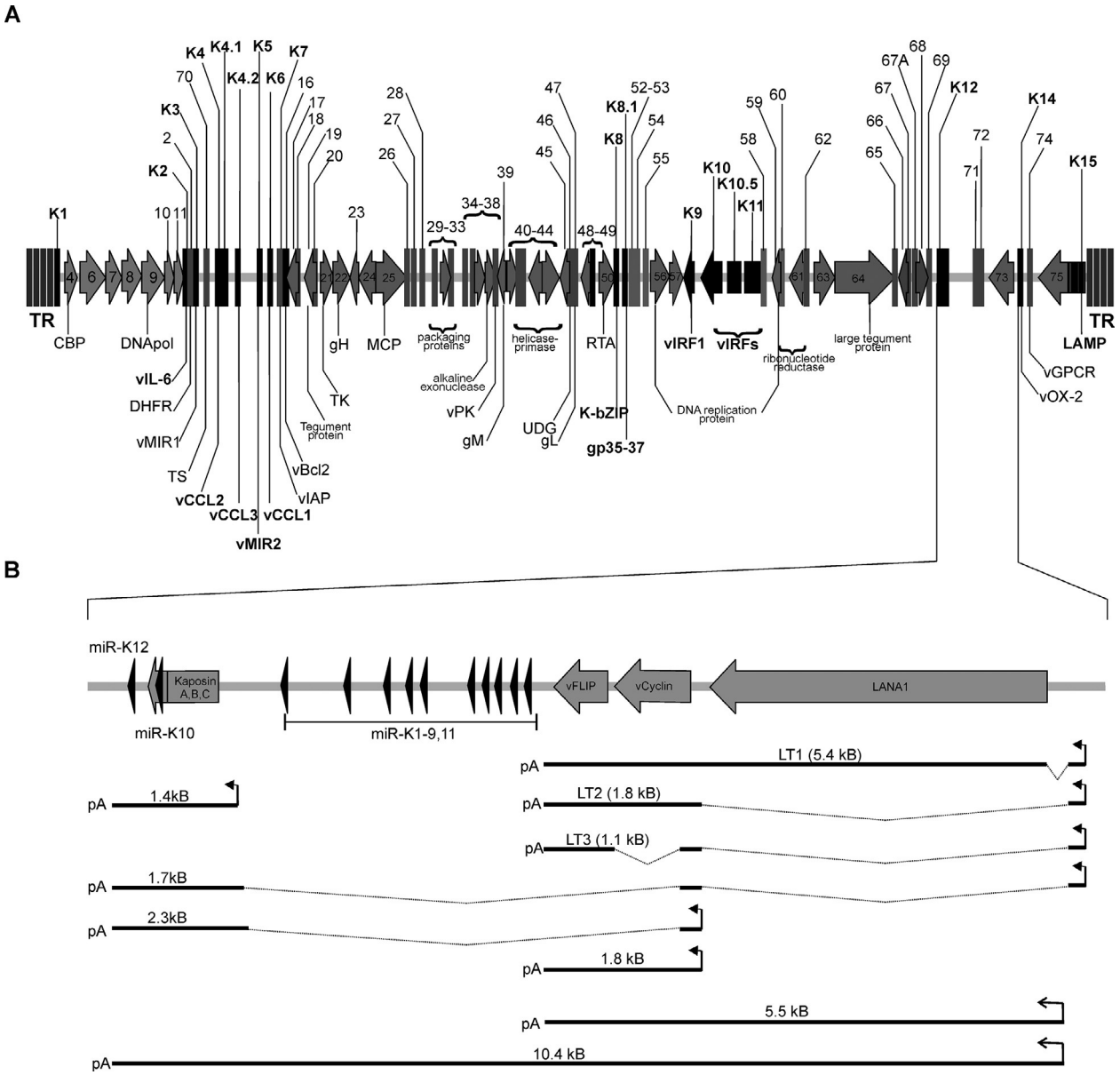

Figure 2 The KSHV genome organization. (a) KSHV comprises a unique long region (LUR) encoding the viral genes which is flanked with multiple repeat units of 801 bp with no coding capacity (TR, dark gray). LUR harbors ORFs that are conserved between KSHV and other herpesviruses, labeled with numbers from 1 to 75 (gray) and KSHV-specific genes annotated with the prefix K that are numbered from K1 to K15 (black). (b) Latency locus (adapted from Russo et al., 1996) contains the four latent ORFs are shown by light-gray boxes with arrowheads and the miRNA cluster (black arrow heads) that are expressed through alternative splicing. The initiation sites of latent transcription are indicated by black arrows and lytic transcription initiation is indicated by open arrows.

All of these proteins have recognizable sequence similarity to, and appear to act in the same regulatory pathways as their human counterparts. Critically however, viral homologs have been generally shown to escape from normal regulatory controls imposed on their corresponding human protein. vCYC, for example, targets the retinoblastoma protein for phosphorylation by recruiting cellular cyclin-dependent kinases. This allows the viral protein to initiate unscheduled S-phase entry of the cell cycle. However, vCYC differs in its resistance to cyclin-dependent kinase inhibitors that control cellular cyclin function. Expression of viral homologs, together with viral proteins that have no cellular counterparts, such as LANA1, is thought to be responsible for the tumorigenic capacity of this virus (**Figure 2(b)**).

While the range in diversity of these homologous nonstructural proteins is unique to KSHV among the human herpesviruses, careful inspection reveals that the functions of these proteins are similar to those found in other viruses. For example, a close functional correspondence between latent EBV proteins and latent KSHV proteins exists despite the fact that there is little or no

sequence similarity. And in general, EBV nonstructural proteins act as signaling molecules to activate specific cellular pathways, whereas KSHV has pirated members of these pathways to function similarly.

Immune Response and Evasion

The surge in KS among AIDS patients reveals the importance of cellular immunity, particularly CD4+ based immunity, to KS tumor control. Cell-mediated immunity assays have measured cellular immune responses against a number of KSHV protein epitopes, including those found on both structural and latent antigens. Among AIDS patients, these responses closely follow regression of KS tumors during highly active antiretroviral therapy (HAART). KSHV proteins deregulate innate immune responses including natural-killer (NK) cell activation, type I interferon (IFN) response, the complement cascade, toll-like receptors (TLR) and cytokine activity. Furthermore the humoral and cellular arms of adaptive immune response are also affected by KSHV.

KSHV encodes a number of proteins that act to overcome innate immune signaling pathways (Figure 3). KSHV encodes four viral interferon response factors (vIRFs) that impact interferon signaling. vIRF1, 2 and 3 block toll-like receptor (TLR)-mediated IFN signaling by various mechanisms. vIRF1 binds p300 and prevents IRF3 mediated transcriptional activation. vIRF2 leads to IRF3 degradation, blocks type I IFN signaling and reduces the activation of interferon response element (ISRE) promoter. vIRF3 interferes with IRF7 DNA binding activity. Further, vIRF3 modulates MHC II-antigen presentation and vIRF4 targets the expression of cellular IRF4 and myc to facilitate lytic replication. ORF45 interaction with IRF7, and RTA-mediated IRF3 and IRF7 ubiquitination and degradation also impair type I IFN signaling pathway. KSHV ORF4 encodes for complement control protein (KCP) that can inhibit complement function. Damania and colleagues have shown ORF63 protein to be a homolog to human nucleotide binding and oligomerization, leucine-rich repeat protein 1 (NLR1) and block NLR-mediated inflammatory responses. vIL6 binds directly to the signal transducer molecule gp130, independently of gp80 and protects the cells from IFNα mediated cell cyle arrest and apoptosis. ORF10 product RIP inhibits the kinase activities of Jak1 and Tyk1. MIR1 (K3) and MIR2 (K5) act in concert to block NK cell-recognition and killing by downregulating of ICAM-1, AICL and MIC A/B. MICB is also targeted by miR-K12-7. KSHV modulates cytokine signaling through expression of Kaposin B, vGPCR and K1which act on, p38/MK2 and PI3K/Akt/mTOR pathways respectively. Additionally, vFLIP associates with IKK complex and activates NFκB signaling and also inhibits apoptosis and autophagy.

KSHV block both adaptive arms of the immune response by diverse mechanisms (Figure 3). Cytotoxic T-cell responses to KSHV in KS patients are considerably less frequent than in asymptomatic KSHV-seropositive subjects, regardless of CD4+ T-cell count. One possible mechanism is KSHV evasion of MHC I immune processing by expressing two proteins, MIR1 and MIR2, that ubiquitinate MHC class I. MIR2-mediated downregulation of ICAM-1and B7-2 impairs costimulatory signals and efficient T-cell acitvation. Another target of MIR1 and MIR2 is interferon gamma receptor 1 which is important for CD4+ T-cell polarization and the diversity of antiviral response. KSHV encodes three viral chemokine ligands vCCL1-3. Beyond their immunomodulatory function of antagonizing the cellular chemokine receptors and inhibiting Th1 response, vCCLs also exhibit angiogenic properties.

Surprisingly, cellular immunity to the constitutively expressed LANA1 protein is generally low. The corresponding EBV protein, EBNA1, escapes immune surveillance by inhibiting its own proteosomal degradation and retarding its own translational synthesis. The latter effect appears to inhibit the formation of misfolded EBNA1 defective ribosomal products that are rapidly processed into MHC I presented peptides. A similar mechanism seems to be at work for KSHV LANA1. Although the two proteins are not homologous, the nucleotide sequence of a central repeat region of both viral proteins is similar but is frameshifted between the two viruses. It remains to be seen whether the nucleotide sequence or the individual protein sequences are responsible for this immunoevasion effect.

KSHV miRNAs Targeting Cellular and Viral mRNAs

KSHV miRNAs have been found to modulate diverse biological pathways by targeting cellular regulatory genes involved in cell cycle, cellular differentiation and metabolism, apoptosis and angiogenesis but also lytic and latent phases of viral life cycle.

KSHV encodes 12 pre-miRNA genes. Gottwein and colleagues reported that KSHV targets many different cellular mRNAs by one or multiple viral miRNAs to affect host survivial pathways. For instance THBS1, an angiogenesis inhibitor, is targeted by several KSHV miRNAs including miR-K1, -3, -6 and -11. miR-K12-1 also suppresses growth arrest by targeting p21 and affects NFκB activation through NFKBIA. There are several viral targets of KSHV miRNAs as well. miR-K12-9 together with miR-K12-5 target RTA to maintain latency and are essential for controlling KSHV replication and latency.

KSHV-Associated Diseases

Kaposi's Sarcoma

KS tumors from all sites and geographic settings are nearly universally infected with KSHV. While KS in the past has been split into categories depending on the epidemiologic setting, such as endemic KS, classical KS, iatrogenic KS, and epidemic KS, all forms of

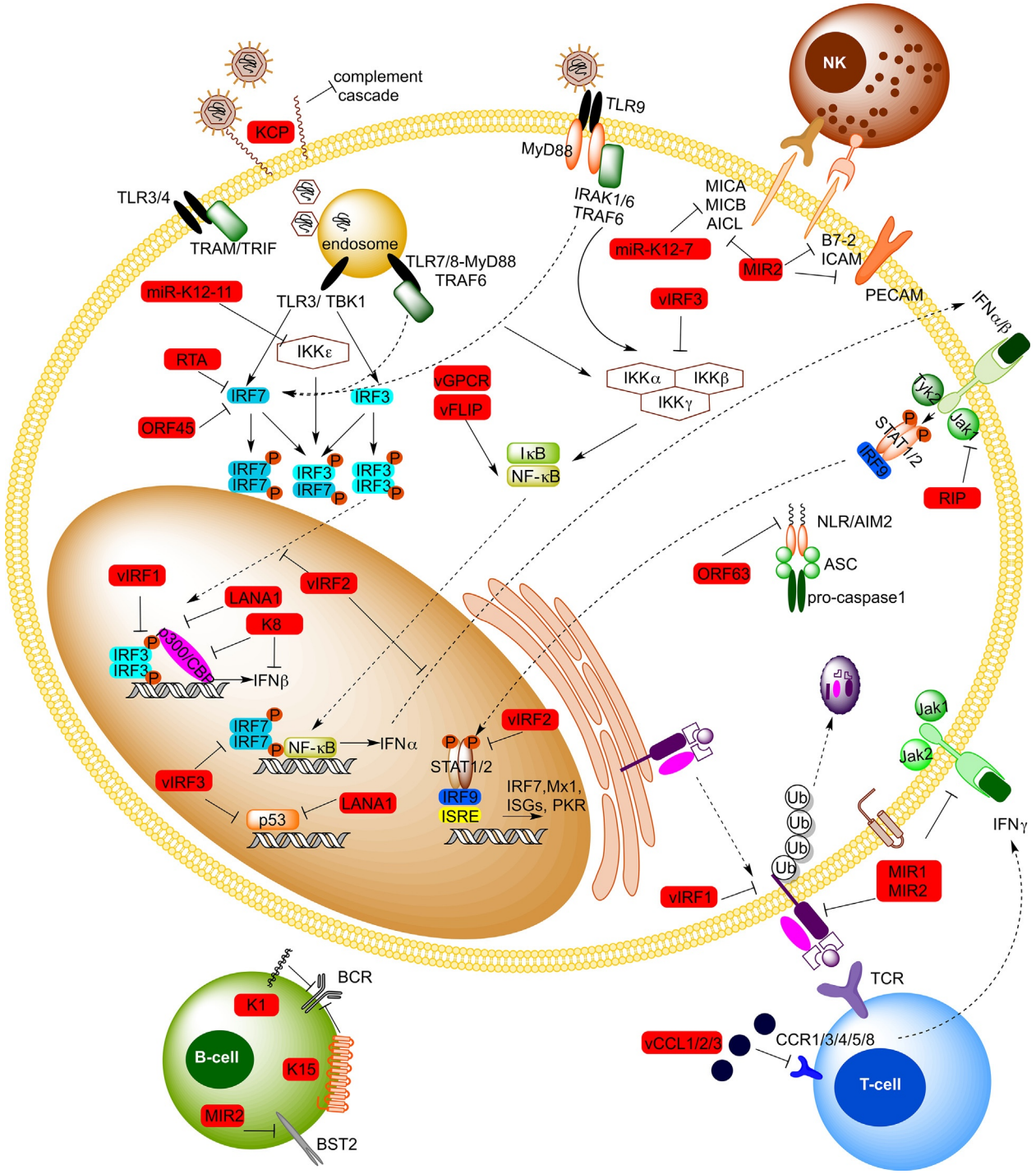

Figure 3 Immune evasion by KSHV. Summary of mechanisms of the innate and adaptive immune response modulation by KSHV.

the tumor are pathologically indistinguishable. Cellular immune status appears to be key to the clinical expression of the tumor among infected persons. In the absence of HIV, KS primarily occurs in the extreme elderly and patients on immunosuppressive therapy. The incubation period from infection to KS has been reported to average 3 years among HIV/AIDS patients, but this appears to be determined by the degree of underlying immunosuppression at time of infection. While KSHV is largely asymptomatic in immunologically intact individuals, it leads to a very high degree of KS clinical penetrance among those who are immunosuppressed. Up to half of early AIDS patients develop KS. On the other hand among transplant patients infected with KSHV 20–60% have been reported to develop clinical disease. Antiherpesvirus drugs, such as ganciclovir, are effective in preventing KS but, once tumors are established, have little impact on disease.

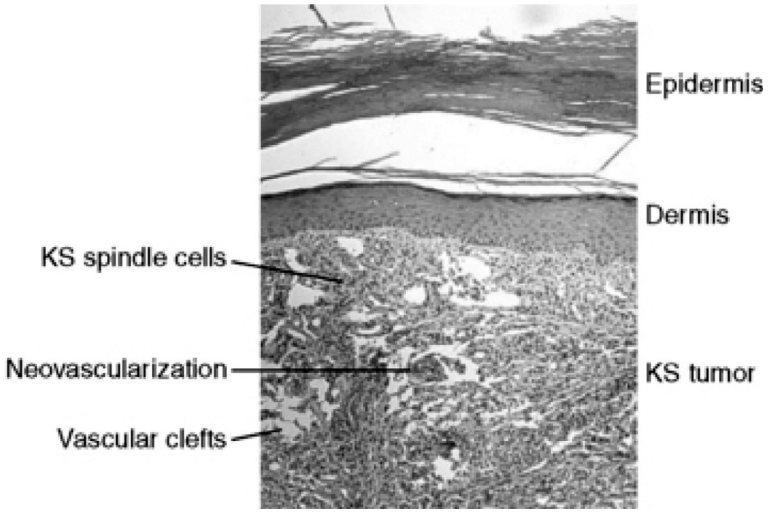

Figure 4 Photomicrograph of a KS tumor showing spindle tumor cells forming irregular vascular clefts that fill with blood. While near-universal KSHV infection of spindle cells is generally seen, the virus is generally absent from the neovascularization that can also be present in tumors. Frequently an inflammatory infiltrate is also seen in KS tumors.

KS tumors express molecular markers suggesting histogenesis from lymphatic endothelium (Figure 4). Microscopically, the tumor is characterized by tumor spindle cells forming disorganized vascular clefts filled with blood cells, giving the tumor a bruise-like appearance clinically. KSHV is detected in virtually all spindle cells, but tumors also show active neoangiogenesis in which more organized vessels lace the tumor mass. Data suggest that KS tumors manifest a spectrum of hyperplastic and neoplastic characteristics. Cellular monoclonality and studies of viral TRs suggest that KS lesions evolve from polyclonal populations of infected spindle cells which may gradually evolve into monoclonal or oligoclonal entities over time.

In KS tumor cells, KSHV virus drives cell proliferation through intracellular expression of a combination of viral oncoproteins (discussed above). While the overwhelming majority of tumor cells have been demonstrated to have a tight viral latency expression pattern, scattered tumor cells clearly show expression of type II viral proteins and data indicates this class of proteins (vIRFs, vIL6, vCCLs, vGPCR and K15) as well as the strictly latent type I class of viral proteins (LANA1, vCYC, vFLIP and miRNAs) contribute to the KS transformation phenotype. Lytically infected cells have been shown to producing VEGF, PDGF, IL6, IL8 and ANGPT2, which can mediate angiogenesis and supports the notion that paracrine stimulation of latently infected cells occurs.

Primary Effusion Lymphoma

PEL is a rare, post-germinal center B-cell tumor accounting for less than 3% of AIDS-associated B-cell lymphomas from various clinical series in developed countries. This lymphoma predominately manifests as malignant effusions in serous body cavities, and generally has an aggressive course among AIDS patients. Unlike KS, PELs are monoclonal and contain high copy numbers of viral genomes. Tumor cells lack most B-cell markers, although V(D)J rearrangement studies confirm their B-cell origin and they express syndecan-1. PEL tumor cells have been reported to be autocrine-dependent on human IL10 and viral IL6. The latter cytokine is peculiar since it appears to form a 'xenocrine' loop in which vIL6 signaling abrogates cellular interferon responses resulting from virus infection that would otherwise stop growth of the tumor cell. PEL cells can be grown stably in culture with each cell maintaining 40–150 copies of the viral episome. Cell lines derived from PEL tumors, particularly from AIDS patients, frequently have EBV coinfection, but EBV-negative, KSHV-positive tumors are also found.

Multicentric Castleman's Disease

Unlike PEL, MCD is considered a hyperproliferative lymphoid disorder, with affected individuals displaying a high rate for subsequent development of other lymphoid malignancies. Among HIV-negative persons, only about 50% of MCD tumors show evidence of KSHV infection whereas $>90\%$ of HIV-positive MCD patients have KSHV present. Studies by Yarchoan and colleagues reveal that MCD virus-derived and cellular IL-6 contribute to B-cell hyperplasia in MCD.

The primary characteristic of MCD is the formation of adventitious germinal centers with marked hyperplasia in lymphoid organs. Examination of these KSHV-positive tumors reveals scattered KSHV-infected cells in the marginal zone of the germinal centers, with the bulk of the cells being uninfected. Aberrant IL6 signaling has been demonstrated to be central to the pathogenesis of MCD, and KSHV-positive tumors have high expression of vIL6, with little human IL6 expression, while KSHV-negative tumors show extensive overexpression of the human cytokine.

Unlike KS and PEL, KSHV-infected cells in MCD show a broad range of viral gene expression and perhaps full lytic viral replication. While standard treatment of KSHV-positive tumors has a high mortality, recent studies have reported success in treatment using anti-CD20 antibodies together with ganciclovir and combination trials of acyclovir with ganciclovir.

Other KSHV-Related Disorders

Post-transplantation primary KSHV infections and or reactivations are associated with fatal, neoplastic and non-neoplastic diseases ranging from KS, PEL, MCD to plasmacytic lymphoproliferative disorders, bone marrow failure and peripheral cytopenias. The extent and consequences of these nonmalignant complications are unknown.

KSHV has been reported to be associated with a number of other malignant and autoimmune diseases, including multiple myeloma, sarcoidosis, post-transplant skin tumors, and idiopathic pulmonary hypertension. Despite initial enthusiasm, subsequent studies have largely failed to demonstrate any association between the virus and these diseases. It remains formally possible that KSHV causes a subset of one or more of these conditions with the majority of disease cases caused by other agents or conditions.

Diagnosis, Treatment, and Prevention

At present, there are no clinically certified tests to detect KSHV infection, although individual research laboratories have the capacity to test samples under special conditions. This presents a major public health problem, particularly in the setting of transplantation and in blood transfusions in highly endemic areas of the world.

Treatment of KSHV-related diseases generally does not directly target underlying viral infection, but instead relies largely on traditional surgical and non-specific chemotherapeutic intervention, and varies on clinical setting. Blinded clinical trials among high-risk AIDS patients show that antiherpesviral treatments such as cidofovir, foscarnet or ganciclovir, have been highly effective in preventing KS. These therapies have also been effective in posttransplantation patients with high viral loads by reducing viremia and virus replication. Current therapeutic options for posttranspantation KSHV-related diseases involve modification or discontinuation of immunosuppresive therapy and substitution of mTOR inhibitors (sirolimus) for anti-calcineurins. Combinatory CHOP-based therapy (cyclophosphamide, doxorubicin, vincristine, prednisone) remains the pivotal therapeutic regimen for posttranasplantation lymphoproliferative disorders and MCDs. For HIV-positive patients additional anti-CD20 treatment is promising. The introduction of HAART therapy has dramatically reduced the risk of KS among AIDS patients, however, since HAART does not target KSHV, it is expected that a resurgence of KS may occur with aging of the AIDS population.

For disseminated disease treatment, combination of HAART with local radiotherapy or systemic chemotherapeutic regimens including liposomal daunorubicin is useful. Emerging approaches aim to inhibit autocrine and paracrine mechanisms by targeting angiogenesis, vascular growth, and cell proliferation (VEGFA, vFlip-IKKg, DLL4, ANGPT2)

There is currently no pursuit of the development of vaccines that might be useful in developing countries. The exquisite sensitivity of KSHV-associated tumors to immune reconstitution and the loss of KSHV from developed populations throughout the world provides evidence that an appropriate vaccine might be extremely effective in controlling this virus infection.

In summary, the public health importance of this virus is clear: KS is the most common malignancy in sub-Saharan Africa and remains an important cause of cancer death among transplant and immunocompromised patients. The wealth of basic science data available for this virus has yet to be used in a significant fashion to prevent or treat diseases caused by KSHV.

Further Reading

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