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KSHV and Kaposi's Sarcoma: The End of the Beginning?

Minireview

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It is now nearly three years since the seminal identification of the genome of a novel human herpesvirus in Kaposi's sarcoma (KS) specimens by Yuan Chang and colleagues (Chang et al., 1994). This discovery changed the face of KS research and ignited a passionate debate about the role of this agent in the etiology of the tumor. Since then, progress in both laboratory and clinical investigation has been swift, and both lines of work are converging on the view that the KS-associated herpesvirus (KSHV; also known by its formal taxonomic designation as human herpesvirus 8) plays a critical role in the development of this neoplasm. Here we summarize the evidence linking virus and disease and consider some of the molecular mechanisms that might underly this association.

Backdrop: KS Epidemiology and Pathogenesis

KS is a peculiar neoplasm that differs from more common tumors in many respects. Histologically, the lesions contain many different cell types, with the dominant cell being the so-called spindle cell, thought to be of endothelial origin. In addition, the tumors contain infiltrating inflammatory cells as well as a profusion of highly characteristic neovascular elements. In immunocompetent hosts, KS is an indolent, largely local process, properties suggestive of a low malignant potential. While often more widespread in immunosuppressed hosts, in whom it can be disfiguring or even fatal, even partial restoration of immune competence can result in arrest (and sometimes remission) of the disease—again distinguishing it from more aggressive neoplasms.

Much of what we know about the histogenesis of KS we owe to pioneering studies by Gallo and colleagues, who first developed reproducible systems for the cultivation of spindle-like cells and established that these cells, while not fully transformed, elaborate a variety of proinflammatory and angiogenic factors (reviewed in Ensoli et al., 1991). Based on these and other in vitro studies, it is now believed that spindle cells are the driving force of KS pathogenesis, with their products responsible for the recruitment of the remaining cell types in a paracrine fashion. If so, then the key etiologic question is: what initiates and sustains spindle cell growth?

The strong association of KS with AIDS naturally led to early efforts to link HIV to KS etiology. However, spindle cells are not infected with HIV, and epidemiologic data suggest that HIV is unlikely to be the sole factor in KS development (Beral et al., 1990). HIV-negative KS, while rare in the West, is common in Africa and long antedated the AIDS epidemic there. Moreover, even among US and European AIDS patients KS is not uniformly distributed: the disease is 7- to 15-fold more common in HIV-positive gay men than in other groups who acquire HIV by nonsexual routes (e.g., hemophiliacs). These and other data suggest that another agent or cofactor, likely sexually transmitted, is also involved in KS etiology.

Enter KSHV

Motivated by these considerations, Chang et al. (1994) searched for DNA sequences that were present in KS lesions and absent in uninvolved tissues. This search yielded two small fragments of DNA that showed clear homology to known herpesvirus sequences. From these starting bits of DNA, the whole 170 kb genome has now been cloned in several laboratories; the complete sequence of one isolate has been published (Russo et al., 1996) and that of a second is nearing completion (see Niepel et al., 1997). The sequence reveals KSHV to be a member of the herpes family's lymphotropic subgroup, whose best-known member is Epstein-Barr virus (EBV).

Not long after the first sighting of the genome, a B cell line (BCBL-1) was identified in which the KSHV genome is present in a latent state; treatment of these cells with phorbol esters induces dramatic lytic replication (Renne et al., 1996). This and subsequently identified similar lines allow high-level virus production, examination of the activity of antiviral drugs, and experimental access to the molecular biology of both latent and lytic infection. In addition, such lines have also served as sources of viral antigens for use in seroepidemiologic studies. Virus from these lines and from primary KS specimens can be transmitted to several other cultured cell lines, but this transmission is extremely inefficient (cf. Foreman et al., 1997). Passage to animal hosts has been similarly difficult: virus from BCBL-1 cells can be transmitted to Rhesus macaques, but again infection occurs at an exceedingly low level and no disease is induced in the recipients.

PCR-based studies on human tissues show that viral genomes are found in virtually all KS tumors, irrespective of the stage of the lesion or the presence or absence of HIV. KSHV genomes have also been strongly linked to several other proliferative lesions: certain AIDS-related B cell lymphomas (these tumors were in fact the source of the B cell lines described above) and a rare lymphoproliferative process called Castleman's disease (Cesarman et al., 1995; Soulier et al., 1995). More recent studies have also proposed an indirect link with multiple myeloma, though the tumor cells themselves appear to be virus-free (Rettig et al., 1997).

Unlike HIV, KSHV can directly infect the KS spindle cell: in situ hybridization studies show viral DNA and transcripts in the vast majority of spindle cells in KS lesions (Boshoff et al., 1995; Staskus et al., 1997). Analysis of the patterns of viral transcription shows that, as expected, most such cells are latently infected. However, in AIDS-KS patients ca. 1%–5% of spindle cells appear to be supporting the lytic cycle (Staskus et al., 1997). In 30%–50% of AIDS-KS patients, viral DNA is also found in circulating B cells (Whitby et al., 1995). Infected AIDS patients frequently display virions in their

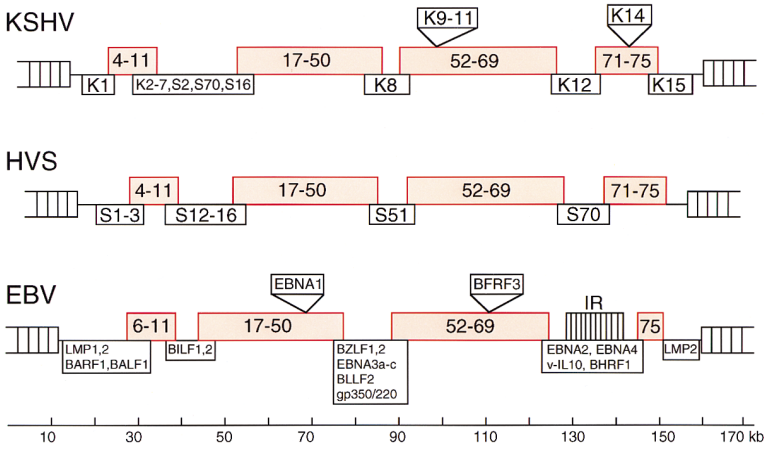

Figure 1. Schematic Depiction of the Genome of KSHV and Related Herpesviruses

Conserved coding regions, largely encoding lytic cycle genes, are shown in solid boxes. Genes unique to each virus are shown in open boxes; ORFs starting with S are largely unique to HVS; those beginning with K largely unique to KSHV. (This figure is modified from Virgin et al., 1997, with the authors' permission.)

saliva (Koelle et al., 1997), suggesting the possibility of productive infection in salivary glands and/or pharygeal lymphoid or epithelial tissue; the epidemiologic significance of such virus, however, is uncertain. Virus is only occasionally found in semen, though such shedding may be low-level and intermittent.

Epidemiologic Studies Strengthen the Link

The above studies locate KSHV squarely at the scene of the crime, but (as students of Perry Mason and O. J. Simpson well know) such information does not suffice to achieve a conviction. Further incriminating evidence, however, has been forthcoming from seroepidemiologic studies. A variety of serolgic tests for KSHV infection have been developed (see Ganem, 1996, for references and review). Most involve detection of a latency-associated nuclear antigen (LANA) present in infected B cell cultures; other tests have examined reactivity to lytic-cycle antigens. The tests for anti-LANA are highly specific but only about 80% sensitive for detection of infection in KS patients, so prevalence figures based on this test must be considered minimal estimates. Around 1%–2% of healthy, HIV-negative US blood donors are positive for anti-LANA; by contrast, 25%–30% of HIV-positive gay men are seropositive in this test. Remarkably, HIV-positive groups known to be at lower risk for KS have dramatically lower rates of anti-LANA positivity: 2%–3% of hemophiliacs, 3%–4% of HIV-positive women. These numbers are strikingly similar to the prevalences of KS in AIDS patients from such groups. Although data on vertical transmission are very limited, they likewise are in accord with the known low prevalence of KS in pediatric AIDS. By contrast, anti-LANA prevalences are high in Africa, where endemic KS has been known to exist for many years. Thus, at the population level there is a strong linkage of KSHV infection with KS risk.

This is not to imply that KSHV seroepidemiology is without controversy. While the use of several different tests for antibodies to lytic antigens has yielded prevalence estimates similar to those revealed by anti-LANA testing, one study (Lennette et al., 1996) has suggested that anti-lytic cycle antibodies might be present in a much higher fraction of the population: up to 25% of HIV-negative donors, and virtually all HIV-positive gay men. Even this study, however, consistently found KSHV infection to be more common in groups at high KS risk than in those at lower risk.

Thus, by every available criterion, KSHV appears to be the agent predicted by the epidemiology of KS: infection precedes development of the tumor, tracks tightly with KS risk, and specifically targets the cell thought to be at the heart of the lesion. This is a very strong case, and most workers in the field now firmly believe that KSHV is necessary for KS development. Formally, further evidence in favor of this could come from either transmission of both infection and disease to a suitable animal or demonstration that a specific intervention (e.g., vaccination) that blocks infection reduces the incidence of the disease in man, but neither result is just around the corner.

But is KSHV sufficient for KS development? Almost assuredly not. Recall that at least 1%–2% of the general population is seropositive, indicating past exposure and ongoing latency in at least some cells of the host. Since these individuals have no measureable risk of KS, other cofactors must be involved, of which HIV infection is the most obvious and (quantitatively) the most important. Deciphering exactly how HIV exerts its effects continues to represent a major charge to the field—and one that, lamentably, has been somewhat underemphasized lately in the face of the explosion of interest in KSHV. The immune deficiency induced by HIV is surely one contributing factor here, but it remains to be seen if HIV gene products make a more specific contribution to KS pathogenesis.

The KSHV Sequence: A Provocative Harvest, but Can We Yet Tell the Wheat from the Chaff?

The upcoming chapter in KSHV research, which began with the publication of the viral genomic sequence, promises to be a page-turner. The sequence, as expected, contains the usual complement of lytic-cycle genes: structural proteins, enzymes involved in DNA synthesis, etc. As depicted in Figure 1, these conserved genes are arrayed in several blocks; flanking them are clusters of novel open reading frames, most of which are not found in previously described herpesviruses. At present there are at least 15 such genes unique to KSHV, designated simply as open reading frames (ORFs) K1–15; a few other genes of this class are shared only with

Table 1. Selected Accessory Genes of KSHV

Viral Gene	Cellular Homolog	Putative Function
K1	—	signaling; growth control?
4	CD46, CR1,2	complement regulation
K2	IL6	paracrine signaling
K4	CC chemokines	paracrine signaling regulation
K6	CC chemokines	paracrine signaling?
16	bcl-2	regulation of apoptosis
K9	IRF-1	gene regulation; growth control?
K12	—	latent membrane protein?
71	DED domain proteins	regulation of apoptosis
72	cyclin D	growth control?
73	—	LANA; transcription factor?
74	CXC chemokine receptor	signaling
K14	OX2	cell–cell interaction?

its closest relative, herpesvirus saimiri (HVS), a simian T-lymphotropic agent. Notably, none of the genes known to be expressed in EBV latency have homologs in KSHV: the latency program of KSHV is therefore a tabula rasa that will have to be filled in by direct experiment.

Table 1 lists some of these accessory genes and summarizes key features gleaned from their sequence. The most striking feature of the list is the preponderance of homologs of cellular genes, including host genes known to function in cell cycle regulation (cyclin D), control of apoptosis (bcl-2), cell–cell interaction (OX2, an immunoglobulin superfamily member), immunoregulation (complement control proteins), and cytokine signaling (CC chemokines, IL6, CXC chemokine receptors, interferon-regulatory factor 1). Many of these viral genes have been shown to encode proteins that preserve the functions predicted by their homologies: for example, ORF 72, the viral cyclin, can bind and activate cdk-6; ORF 16, the viral bcl-2 analog, can block experimental apoptosis; and ORF K2, the viral IL6 homolog, can support the survival of IL6-dependent cell lines (see Moore et al., 1996, and Niepel et al., 1997, for references). Sometimes the viral products display unexpected activities: for example, the virus-encoded chemokine receptor homolog (ORF 74) appears to be constitutively active, signaling even in the absence of exogenous ligands (Arvanitakis et al., 1997), while one of the chemokine homologs (ORF K4) appears to be able to function as an antagonist of chemokine signaling (Kledal et al., 1997).

The existence of virally encoded cytokines and cytokine receptor genes in a tumor in which paracrine signaling has long been suspected is particularly provocative. But we are deeply in the dark as to whether and how such genes might function during pathogenesis, and the temptation to jump to facile conclusions should be resisted. For example, the patterns of expression of some of these genes (e.g. ORFs K4 and 6) suggest that they may be expressed primarily in the lytic cycle rather than in latency, and it is latency-associated genes that are primarily responsible for tumorigenesis in other oncogenic herpesviruses. Is it possible that lytically infected cells might elaborate factors that support tumorigenesis by surrounding cells? Perhaps, but this model is complicated by the fact that most surrounding spindle cells are latently infected, seemingly implying that latency itself might be required for receptivity to such signals. On the other hand, plausible roles in the lytic cycle for many of the genes in Table 1 can be readily envisioned: viral inhibitors of apoptosis, for example, may be required to prevent premature host cell death until the virus can complete its lytic cycle. So the sequence should serve as a stimulus and not as a substitute for the next, less glamorous phase of KSHV research: the enumeration and characterization of the viral genes expressed in latency.

Already there has been excellent headway made here. The most abundant latent transcript harbors a 60 codon open reading frame (ORF K12) that could encode an extremely hydrophobic small polypeptide of as-yet-unknown function. LANA, the latent nuclear antigen so useful in serologic work, has been identified in several labs as the product of ORF 73; its sequence suggests that it may represent a transcription factor. Of the genes in Table 1 that have cellular homologs, only that for ORF 72 (v-cyclin) is known to be expressed in latency, though many of the others have not yet been closely examined and may yet qualify.

Another approach to the identification of genes that may relate to KS pathogenesis has been the search for genes that promote growth deregulation in cultured fibroblasts or other heterologous model systems. To date, such work, now ongoing in several laboratories, has fingered additional suspects from the list in Table 1, and efforts are underway to determine the significance of these findings for tumorigenesis in vivo. It will be important to determine, for example, whether any of these genes are expressed in latently infected KS spindle cells. Even assuming that they are, it is not clear how to relate overt cell transformation in these heterologous systems to the rather more subtle growth deregulation of spindle cells in a KS tumor. Perhaps the latter is the result of low-level expresssion of these genes or their modulation by other viral or host genes. Or perhaps these genes relate not to KS but to the rarer, more overt lymphoid malignancies also linked to KSHV. Time will tell, but one could not imagine a more promising start for this line of inquiry.

Perspective

The rapid pace of progress in KSHV research in its first 3 years has given us a broad overview of the biology of the virus and generated the experimental systems necessary for a concerted molecular attack on its pathogenetic mechanisms. The year 1997 thus marks an important way station in the evolution of the field: in

Churchill's memorable phrase, we have arrived at the end of the beginning. Now comes the spade work: for the virologist, this means enumerating the latent genes, working out the biochemical activities of their products, developing better systems for infectivity testing, and characterizing the determinants of immunity. Major challenges here will be to determine whether the latency program in B cells differs from that in KS spindle cells and whether any of the products of the lytic cycle can also influence the growth of latently infected or uninfected cells. For the clinical investigator, it means defining more precisely the routes of transmission and determining whether any other clinical syndromes are attributable to infection. Detail work, to be sure, but, as Mies van der Rohe famously noted, God dwells in the details. Given the highly unusual biology of KS, it is likely that the details of KSHV pathogenesis will conform only loosely to other precedents in viral oncology and may well suggest entirely novel mechanisms that could form the basis for new treatment or prevention strategies for KSHV-associated diseases.

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