Initiation of angiogenic Kaposi's sarcoma lesions

A new mouse model reported in this issue of *Cancer Cell* implies that VEGF/KDR-mediated paracrine effects induced by the lytic cycle vGPCR signaling protein encoded by human Kaposi's sarcoma-associated herpesvirus (KSHV or HHV8) may be involved in promoting the proliferation of the KSHV latently infected spindle endothelial cells of Kaposi's sarcoma.

One of the first hallmarks noted by the CDC at the beginning of what became the AIDS epidemic in the United States was the sudden unusual occurrence of cancerous red skin lesions in a cohort of young homosexual men in New York and San Francisco. These angiogenic lesions known as Kaposi's sarcoma (KS) resembled those in rare cases (defined originally in 1870) in elderly Mediterranean Jewish men and also those in a later, more aggressive version in Central Africa (starting in the 1950s). We now appreciate that all forms of KS (epidemic, classic, introgenic, and endemic) are associated with infections by a novel herpesvirus known as KSHV or HHV8 discovered in KS lesion tissue in 1994 (Chang et al., 1994). This is normally an extremely rare disease correlating with low seropositivity rates in most parts of the world, except sub-Saharan Africa (60%) and the Mediterranean (5% to 20%). However, the rates of KS disease increase up to 500-fold in solid organ transplant patients and up to 20,000-fold in male homosexual AIDS patients. The huge increase in KS in Southern Africa, where it is now the most frequently encountered cancer, represents a combination of the introduction of novel HIV infection-related immunosuppression with a population that was already almost ubiquitously but asymptomatically infected by KSHV. Nevertheless, it is very evident that HIV alone does not cause KS, whereas KSHV is essential for KS lesions to form (Jenner and Boshoff, 2002).

There is no clear explanation of why HIV infection specifically depresses normal cell-mediated immunological control of KSHV, which is presumed to be dormant in latently infected B cells and perhaps other myeloid or endothelial cells (EC); however, dysregulation of immune cytokine and chemokines and perhaps the Tat protein is presumed to lead to reactivation and spread of KSHV to dermal vascular or lymphatic endothelial cells, which then convert into the characteristic spindle cell phenotype and proliferate as an unusual viral-driven neoplasia with angiogenic characteristics. In late stage nodular or disseminated KS, essentially all spindle cells are latently infected by KSHV and express just the viral LANA-1 protein and probably also the vCyc-D and vFLIP proteins (Dupin et al., 1999; Jenner and Boshoff, 2002). LANA-1 is a chromatin-interacting repressor that binds to the multicopy episomal viral genomes giving a distinctive punctate nuclear pattern. It probably plays a role in both segregation of progeny genomes and cell growth control. Both LANA-1 and vCyc-D have promitotic properties, and vFLIP has anti-apoptotic activity as well as upregulating NF_KB, a key feature of several rare KSHV-associated lymphomas such as primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD) that also occur at increased frequency in AIDS patients. Infectious KSHV virions derived from TPA-treated PEL cell lines can be used to infect cultured normal primary human dermal microvascular endothelial cells (DMVEC) to produce LANA-1-positive latently infected cells with a proliferating spindle cell phenotype that closely resembles KS (Ciufo et al., 2001).

Unlike HIV, KSHV is clearly an ancient human virus that has coevolved with its migrating human host since at least the origin of modern humans in Africa, and almost certainly throughout mammalian evolution, judged by the presence of closely related viruses in chimpanzees and in a number of other old world primate species. However, unlike with other more typical and ubiquitous human herpesviruses, KSHV infection (presumed to be through saliva) is apparently relatively inefficient and may not have had time to catch up with the relatively recent rapid expansion of human populations (outside of Africa) after migration to new continents or from the Ice Age refuges in Europe and Asia (Zong et al., 2002).

Early stage KS lesions, particularly those that fit into the original description of patch and plaque types, rather than nodular or invasive, tend to have many fewer spindle and LANA-1-positive cells and include apoptotic cells (Sturzl et al., 1999). Most studies of clonality in KS lesions have concluded that they are either mixtures of oligoclonal cells or at best only partially monoclonal, suggesting that whereas some early development may involve spread of infection to new cells, the later nodular and invasive stages probably involve cladal expansion of proliferating populations that are now virtually totally infected and lack an apoptotic subset.

The two great puzzles of KSHV oncogenesis are, firstly, considering that KSHV clearly has the potential for causing neoplastic lesions and all KS lesions have KSHV in them, why is KS so rare in the absence of HIV? And secondly, how can one rationalize why virtually all of the unexpectedly large number of KSHV genes that have been shown to have some form of in vitro transforming or potentially oncogenic properties fall into the lytic cycle class rather than being latent state genes?

In this issue of *Cancer Cell*, Montaner and colleagues (2003) make the best case yet that, despite being a classic lytic cycle gene product, the virally encoded G protein-coupled receptor (*vGPCR* or *ORF74*) gene is indeed the predominant oncogene of KSHV. Most investigators have agreed that in isolation, the vGPCR protein, as a "captured" relative of the cellular IL8 (α-chemokine) receptor, has all of the right characteristics, including constitutive (or ligand-independent) MAPK signaling and pro-angiogenic properties to explain the vascular appearance of KS lesions. As the laboratories of Mesri and Cesarman have elegantly demonstrated, stable ectopically expressed vGPCR induces focus formation in NIH-3T3 cells, and these cells in turn produce angiogenic lesions resembling KS in nude mice. Serine kinase-mediated intracellular signaling by the vGPCR produces upregulated PI3K/AKT and MAPK/ERK pathways leading to increased AP1 activity and secreted VEGF production and, in EC, to upregulation of the VEGF receptor KDR (FLT-2) (Bais et al., 1998; Cannon et al., 2003).

In the other well-studied γ her-

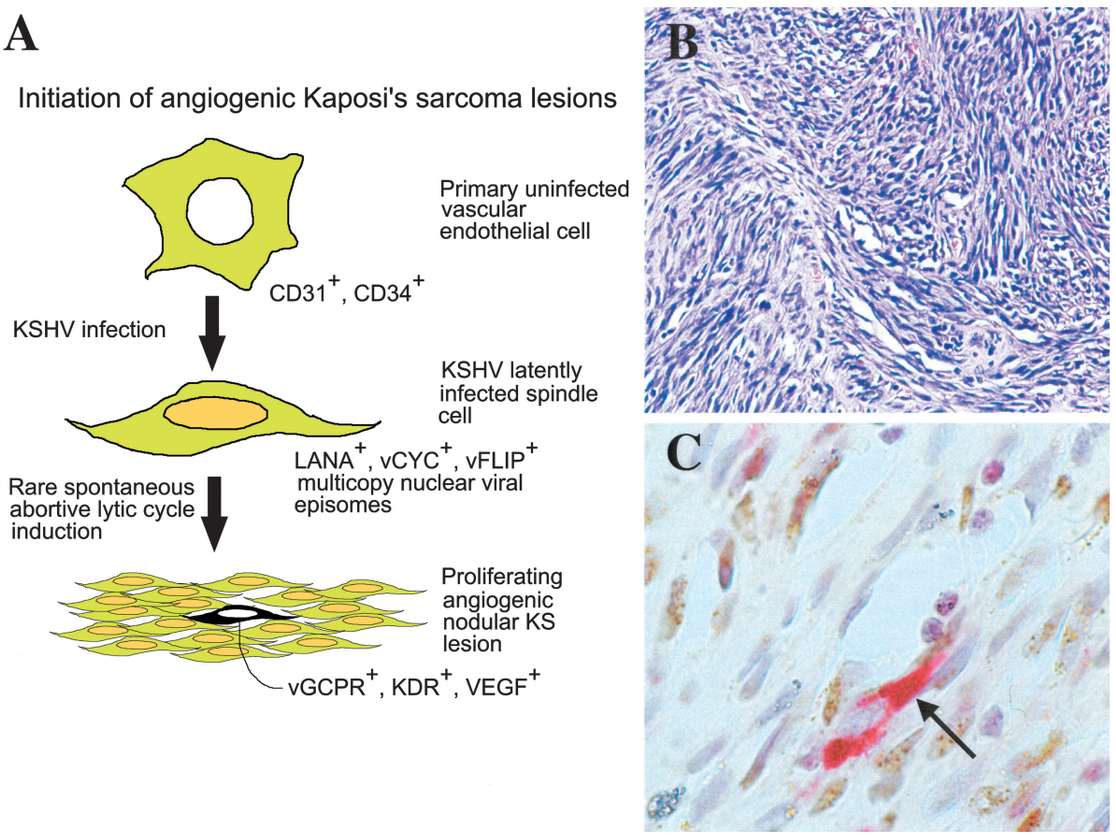

Figure 1. Contributions of both latent and lytic viral proteins to KS pathogenesis

A: Schematic diagram illustrating the current model supported by Montaner et al. (2003) for conversion of endothelial cells (yellow cytoplasm) to elongated spindle cells (LANA-positive nuclei in red) by KSHV latent infection, and the subsequent proliferative neoplasia of Kaposi's sarcoma driven by paracrine effects from sporadic lytic cycle induction of vGPCR protein expression (black cytoplasm).

B: Histological image of a human nodular KS biopsy tissue with large numbers of spindle cells (paraffin section, hematoxylin stain).

C: Double label immunohistochemistry of a human nodular KS biopsy specimen showing both latent state infected LANA-positive spindle cells (brown nuclear spots) and an occasional cell (arrowed) expressing the lytic cycle vGPCR protein (red cytoplasm) (from Chiou et al., 2002).

pesvirus models for oncogenesis, only latency genes are known to be essential as contributors to immortalization of B cells or T cells and for oncogenicity (e.g., *LMP1* and *EBNA2* in Epstein-Barr virus or *STP* in Herpesvirus saimiri). In contrast, KSHV encodes at least five lytic cycle proteins that either behave as oncogenes in focus formation assays (*K1*, *vIRF1*, *vGPCR*) or at least have growth-promoting antiapoptotic or angiogenic properties (*vIL6*, *vMIP*). Because most PELs, MCD, and some KS samples, as well as KSHV-infected cultured DMVEC spindle cells, display a low level of spontaneous lytic cycle gene expression (usually in about 1% of the cells), many authors have speculated that lytic cycle gene expression may contribute to the KS phenotype possibly via paracrine effects. Normally we think of the lytic cycle in herpesviruses as a process that leads to rapid cell death. However, the pattern of lytic cycle expression in both PELs and DMVEC instead suggests a hierarchy of abortive lytic progression in which some cells express only one lytic gene (*vIL6*) or just the earliest set of lytic genes, including the immediate early transcriptional regulators and one or more viral β-chemokines (*vMIP*). Even fewer cells also express the core DNA replication proteins and the late early vGPCR, and often there are very few or no cells at all that express the true late gpK8.1 protein (Chiou et al., 2002; Ciufo et al., 2001). Therefore, some of these abortive lytic stages may still be compatible with cell survival or at least delayed cell death. With regard to the *vGPCR* gene, both the viral mRNA and its isolated upstream promotor display typical PAA-insensitive early lytic cycle properties in both PELs and DMVEC, and are not expressed significantly in the vast majority of otherwise latently infected cells. This applies also to *vGPCR* mRNA and protein expression detected by in situ hybridization or immunohistochemistry in KS lesion samples. However, a small fraction of cells in some KS samples (particularly nodular cases) do reveal typical lytic cycle levels of *vGPCR* mRNA and protein expression (Chiou et al., 2002; Kirshner et al., 1999).

Montaner et al. (2003) have asked whether in vivo retrovirus-mediated infection leading to specific expression of *vGPCR*, *vCYC-D*, *vFLIP*, *vIRF1*, or *vBCL2* genes can produce KS-like

lesions in mice. They found that this was indeed the case, but only for *vGPCR* and not for the other genes in isolation. The authors chose an apparently very successful and elegant approach for targeted expression in vascular EC using a transgenic mouse expressing an avian retrovirus receptor under the control of an EC-specific enhancer domain. Control infections with ALV-*GFP* showed expression only in CD31-positive vascular EC, and the use of ALV expressing wild-type vGPCR but not mutant vGPCR yielded fast growing nodular KS-like vascular lesions at high efficiency. Note that, unlike human KS lesions, these mouse tumors do not contain or express any KSHV latency genes, but curiously just like human KS, PEL, and infected DMVEC, vGPCR expression occurred in just a small fraction of the spindle-like tumor cells. A similar fraction of tumor cells (presumably the same cells) also expressed VEGF and KDR. Most interestingly, when infected EC expressing either GFP or vCyc-D/vFLIP were coinjected together with vGPCR-expressing cells, the former also contributed cooperatively to the mass of the tumor, although they did not proliferate in the absence of vGPCR. Furthermore, tumors that included cells expressing the latency genes (but not those including cells expressing GFP) grew faster than those with vGPCR only. Evidently, the presence of vGPCR-positive cells produced paracrine or bystander effects that promoted the propagation and survival of the cells expressing latency genes, which must already have exhibited some extra level of growth dysregulation over the GFP-only infected cells.

The strange pattern of vGPCR expression in just a small percentage of the mouse tumor cells is unexplained, but is remarkably similar to the situation in human KS, and even to that in another about to be published study in which transgenic mice receiving a *vGPCR* gene under the control of the SV40-enhancer again produced KS-like skin EC lesions with confirmed vGPCR expression in just a small fraction of the tumor spindle cells (M. Reitz, personal communication). Whether or not the occasional cells that express vGPCR survive long-term, these results create an attractive scenario in which KS development involves initial latent infection of normal vascular EC, which converts them into spindle cells, followed by a proliferative phase requiring expression of the early lytic cycle vGPCR protein in at least a few spindle cells, with subsequent activation of a VEGF/KDR autocrine loop plus apparent paracrine effects on adjacent spindle cells that are only latently infected (Figure 1).

This experimental model offers the possibility of additional exciting developments in the future such as asking whether including LANA-1 or vMIP or even HIV TAT in vGPCR-driven tumors contributes to tumor progression. Although, in contrast to its cellular counterparts such as CCR1, vGPCR evidently has acquired the novel ability to signal in a ligand-independent manner, this signaling can still be up or down-regulated by certain α-chemokine ligands such as GROα and IP10, respectively (Gershengorn et al., 1998). If indeed the growth of the mouse KS-like tumors can be manipulated positively or negatively by GROα or IP10, this could provide further strong support for a key role of vGPCR signaling in KS pathogenesis.

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Selected reading

Bais, C., Santomasso, B., Coso, O., Arvanitakis, L., Raaka, E.G., Gutkind, J.S., Asch, A.S., Cesarman, E., Gerhengorn, M.C., and Mesri, E.A. (1998). Nature *391*, 86–89.

Cannon, M., Philpott, N.J., and Cesarman, E. (2003). J. Virol. *77*, 57–67.

Chang, Y., Cesarman, E., Pessin, M.S., Lee, F., Culpepper, J., Knowles, D.M., and Moore, P.S. (1994). Science *266*, 1865–1869.

Chiou, C.-J., Poole, L., Ciufo, D.M., Cannon, J.S., Alcendor, D.J., Zong, J.-C., Ambinder, R.F., and Hayward, G.S. (2002). J. Virol. *76*, 3421–3439.

Ciufo, D.M., Cannon, J.S., Poole, L.J., Wang, F., Murray, P., Ambinder, R.F., and Hayward, G.S. (2001). J. Virol. *75*, 5614–5626.

Dupin, N., Fisher, C., Kellam, P., Ariad, S., Tulliez, M., Franck, N., van Marck, E., Salmon, D., Gorin, I., Escande, J.-P., et al. (1999). Proc. Natl. Acad. Sci. USA *96*, 4546–4551.

Gershengorn, M.C., Geras-Raaka, E., Varma, A., and Clark-Lewis, I. (1998). J. Clin. Invest. *102*, 1469–1472.

Jenner, R.G., and Boshoff, C. (2002). Biochim. Biophys. Acta *1602*, 1–22.

Kirshner, J.R., Staskus, K., Haase, A., Lagunoff, M., and Ganem, D. (1999). J. Virol. *73*, 6006–6014.

Montaner, S., Sodhi, A., Molinolo, A., Bugge, T., Sawai, E., He, Y., Li, Y., Ray, P., and Gutkind, J.S. (2003). Cancer Cell *3*, this issue, 23–36.

Sturzl, M., Hohenadl, C., Zietz, C., Castanos-Velez, E., Wunderlich, A., Ascherl, G., Biberfeld, P., Monini, P., Browning, P.J., and Ensoli, B. (1999). J. Natl. Cancer Inst. *91*, 1725–1733.

Zong, J.C., Ciufo, D.M., Viscidi, R., Alagiozoglou, L., Tyring, S., Rady, P., Orenstein, J., Boto, W., Kajumbula, H., Romano, N., et al. (2002). J. Clin. Virol. *23*, 119–148.