

KSHV/HHV-8 and HIV infection in Kaposi's sarcoma development

*Pawan Pyakurel¹, Amos. R. Mwakigonja^{1/2}, Fatemeh Pak¹, Ephata Kaaya^{1/2}, Peter Biberfeld¹,

¹Immunopathology Lab., Department of Pathology and Oncology, Karolinska Institute/Hospital, S-171 76 Stockholm, Sweden, ²Muhimbili University College of Health Sciences, P. O. Box 65023, Dar-Es-Salaam, Tanzania,

*Correspondence: Pawan Pyakurel, Immunopathology Laboratory, M9:00, Karolinska Institute/Hospital, S171-76, Stockholm, Sweden. Tel: 46-8-5177 4523. Fax: 46-8-345820. E-mail: pawan.pyakurel@ki.se

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Kaposi's sarcoma

Kaposi's sarcoma first described by Moritz Kaposi in 1872 as "idiopathic multiple pigmented sarcomas of the skin" [1] is an angioproliferative, tumour-like lesion usually developing in the skin [2], and eventually disseminating to multiple cutaneous sites, viscera and lymph nodes. Previously a rare disease, it is now a global health care and clinical problem because of its association with the HIV pandemic [3] and other immunosuppressed states[4].

Four clinically different KS forms are now recognized [5]:

- a) Classical or sporadic KS (CKS), originally described [1] as a slow growing, indolent tumor mostly developing in the extremities of elderly males of eastern and Mediterranean Europe.
- b) Acquired immunodeficiency syndrome (AIDS)-associated KS (AKS), the most frequent tumor of human immunodeficiency virus type I (HIV-1) infection and the most aggressive and rapidly growing form of KS in AIDS, with early dissemination in the skin and viscera.
- c) Endemic KS (EKS), predominant in eastern and central sub-Saharan Africa before the AIDS epidemic and clinically similar to CKS, but also seen in a more fulminant and fatal form in children. The childhood EKS is mostly lymphoglandular with or without skin involvement.
- d) Iatrogenic KS (IKS), seen in drug related immunosuppressed patients, e.g. transplant patients, emphasizing the importance of immune disturbance as a co-factor in the pathogenesis of IKS and AKS, and possibly also EKS.

In spite of the clear clinical differences the histopathology of the various KS forms is essentially the same, with characteristic changes related to stage in the development of the KS tumor[6].

The epidemiology of AKS led to the discovery of a novel herpes virus [7], which subsequently was shown to be associated with all clinico-epidemiological forms of KS [8]. The virus was rapidly characterised as a KS associated herpes virus (KSHV) and classified as human herpes virus type 8 (HHV-8). It was soon recognized to also be associated with some rare types of lymphomas in AIDS patients, namely primary effusion lymphoma or body-cavity-based-lymphoma (PEL/BCBL) and Castleman's disease (MCD)[9].

Human herpesvirus type 8 (HHV-8)

Human herpesvirus 8 or Kaposi's sarcoma associated herpesvirus (HHV-8/KSHV) was recognized to be a novel gamma-2 herpesvirus of the rhadinovirus genus closely related to the human gamma -1 herpesvirus, Epstein-Barr virus (EBV) [10].

A number of viral glycoproteins have been characterized including K8.1A and ORF8 (gB) shown to bind to cell surface heparan sulfate [11] and the cell receptor integrin $\alpha 3\beta 1$,

respectively, thereby mediating virus entry through endocytosis [12]. In KS lesion HHV-8 is predominantly found in so called spindle (SC) cells but also in some lymphocytes, monocytes and keratinocytes [13]. The virus replicates predominantly in a latent form as closed circular episomal DNA [14] within the nucleus of KS tumor cells (SC), B cells of MCD and other infected mononuclear cells [15] or in lytic form. It has been shown that the episomal viral DNA is tethered to metaphase chromosomes and copied in tandem with host cell DNA during cell division [16]. Latent genes well demonstrated in infected KS SC are the latent nuclear antigen (LANA-1) encoded by ORF 73, viral cyclin (v-cyclin) (ORF 72), v-FLIP (ORF 71, K13) and a small membrane protein (kaposin, ORF K12), which are all adjacent in the genome [16]. Lytic virus expression is most frequent in MCD, moderate in KS and relatively rare in PEL cells. Common viral genes found during lytic expression include K1 transmembrane protein, v-GCR (ORF 74), v-IRF (ORF K9), v-IL-6 (ORF K2) and v-MIP (ORF K4.1) [15].

LANA-1

The latency associated nuclear antigen type 1 (LANA-1) protein is the well expressed and highly immunogenic, latent nuclear antigen of HHV-8 considered important in the generation and maintenance of HHV-8 associated malignancies [17]. It can function in cell cycle regulation by competing with E2F for binding of hypophosphorylated pRb thus freeing E2F to activate the transcription of genes involved in cell cycle progression [18]. Aberrant E2F activity can also trigger apoptosis via the p53 pathway but LANA-1 binds p53, repressing its transcriptional activity and ability to induce apoptosis. Therefore the inhibition of p53 by LANA-1 allows latent HHV-8 to promote cell cycle progression whilst inhibiting apoptosis [19].

The LANA-1 antigen can be demonstrated by immunohistochemistry also in formalin fixed paraffin embedded biopsies. It is expressed in most SC of both early and late stage lesions of all different clinical KS forms (AKS, EKS, CKS and IKS) [8, 20] and therefore used as a diagnostic marker in suspected HHV-8 related lesions and for serology in patients of LANA-1 antibodies by immunocytochemistry on infected BCBL cells that gives a characteristic speckled nuclear staining. Several studies have shown an increase in the number of LANA-1 positive cells during progression of KS lesions [21, 22] allowing early detection and quantification of HHV-8 infection in KS lesions.

Pathogenesis of KS

The pathogenesis of KS is still unclear and appears complex, involving various mechanisms dependent on viral and cellular factors related to inflammation, angiogenesis i.e. endothelial growth factors (β -FGF, PDGF, VEGF) including HIV-Tat, anti-apoptosis (vBCL2) and cell proliferation [2, 13, 23, 24]. Characteristic for HHV-8 is the high homology of several viral ORFs with cellular genes suggesting they were pirated from host chromosomes during viral evolution. Some of these genes are involved in down modulating the host immune responsiveness to target infected cells, whereas others modulate cell proliferation, cell differentiation and angiogenesis [13]. They include the vBcl-2, vIL-8R, vMIPs, vIL-6, and the D type viral cyclin [13], whose functions are all closely related to that of their cellular homologs.

HHV-8 infected cells escape immune response targeting by down regulation of surface MHC mediated by two transmembrane proteins, MIR1 and MIR2, encoded in ORFs K3 and K5, respectively [25] (Fig 1). MIR1 and MIR2 remove MHC I from the plasma membrane through enhanced endocytosis, and lysosomal degradation which efficiently inhibits MHC I surface expression (Fig 1). Downregulation of MHC I and its accessory immune receptors poses the risk of initiating a natural killer (NK) cell response by initiating receptor-activated apoptosis through Fas (CD95/Apo-1) in cells lacking appropriate MHC I expression. However, HHV-8 can inhibit NK-mediated killing through expression of anti apoptotic v-FLICE-inhibitory proteins (v-FLIPs) [25]. V-FLIP encoded by ORF K13 (ORF71) possesses death effector domains and acts as a dominant-negative inhibitor of receptor-activated apoptosis by binding to Fas-associated death domain protein and caspase 8 (FLICE) [26]. This prevents activated caspase recruitment into the death-inducing signaling complex (Fig 1).

HHV-8 v-FLIP shares with c-FLIPs the ability to activate NF- κ B through I κ B kinase activation [25] which is essential for the growth and survival of the cell. Our previous studies on KS biopsies have shown that apoptosis clearly decreases during development of early to late nodular KS lesions [27], and that the expression of anti-apoptotic v-FLIP and cellular Bcl-2 increase from early to late stage KS lesions, [27, 28]. Thus viral exploitation of these two anti-apoptotic pathways contributes to the tumor-like growth and progression of the KS lesion.

HHV-8 encoded v-cyclin binds with cyclin dependent kinases (CDK6), and this complex phosphorylates pRb releasing the transcription factor E2F, which in turn, activates the

transcription of S-phase genes (Fig 1). However, unlike complex of cellular cyclin with CDk6, vCyclin-CDK6 complexes are resistant to CDK inhibitory proteins and can even abolish its inhibitory effect by a concomitant destabilization and degradation, which may lead to unregulated cell cycle progression and transformation and thereby promote tumor development [29].

Kaposin, a latency gene encoded by ORF K12 represents a potential viral oncogene and is characterized as a transforming gene [30], although little is known about its role in deregulating cell signalling [31]. It is present in three isoforms Kaposin A, Kaposin B and Kaposin C which are all translated from the ORF K12 region [32]. Kaposin B is expressed by all HHV-8 infected cell and can activate the p38–MK2 pathway [33] (Fig 1). Kaposin B binds to MK2 in the nucleus, which then will be exported to the cytoplasm. The activated cytoplasmic MK2 will then block the degradation of the messenger RNAs transcribing various cytokines necessary for cell survival (normally unstable because they contain AU-rich elements), hence increasing their translation [33]. The Kaposin gene also encodes MicroRNAs (miRNA), small conserved non-coding RNA molecules which regulate expression of genes by binding to the complementary messenger RNAs [34]. The HHV-8 miRNAs are confined to a region of the kaposin gene (ORF K12) [34], which is expressed by SC at all KS stages [35] and which can induce tumorigenic transformation of infected cells [30]. Blocking of this miRNA can thus hinder the function of kaposin, which could be of therapeutically interest [34]. The viral and cellular genes regulated by these HHV-8 miRNAs still remain to be identified.

HHV-8 also encodes a G-protein-coupled receptor (vGCR) homolog to the human angiogenic, chemokine interleukin-8 receptor (IL-8R) [36] (Fig 1). Angiogenic responses induced by vGCR are mediated by upregulation of vascular endothelial growth factor (VEGF) [37]. The constitutive activity of vGCR could therefore have a role for VEGF expression by SC during the development of early stage KS lesions [38]. Furthermore the vGCR dependent expression of autocrine and paracrine growth factors (bFGF, VEGF,) promotes the angiogenesis and edema [23, 39] seen in KS patients. It was also shown [40] that viral envelop glycoprotein gB (ORF8) can activate the VEGFR-3 receptor and trigger receptor signalling on the surface of microvascular endothelial cells, thereby modulating cell migration and proliferation. VEGFR-3 expression and activation may also enhance HHV-8 infection and participate in HHV-8 mediated transformation. Thus VEGFR-3 activation in the endothelium appears to be an important factor in the pathogenesis of Kaposi's sarcoma [40].

Histogenesis of KS

The histopathology of KS is characterized by an early infiltration of mononuclear inflammatory cells, formation of small, irregular, endothelial lined slits around new blood vessels (angiogenesis) and extravasation of erythrocytes [2] with accumulation of hemosiderin pigments. KS at early stages reflects a predominantly reactive cell proliferation of polyclonal nature that can regress, but usually progresses to a nodular possibly clonal tumor [2, 41]. Pathognomonic for KS development from early patch/plaque to late nodular tumor lesions is the increased appearance of so-called spindle cells (SC) expressing CD34 (hematopoietic stem cell and vascular endothelial marker). At the nodular KS stage there is less inflammatory cell infiltration, mostly seen at the periphery of the dense nodular accumulation of SC. These nodular KS skin lesions later may ulcerate. Unlike typical metastatic cancers, KS often appears early as a multicentric tumour, with each lesion arising de novo by a localized development and proliferation of SC [42].

Most SC are positive for CD34 but a considerable number of CD34+ SC are LANA- at all AKS/EKS stages [21, 22]. This apparent heterogeneity in viral permissiveness of CD34+ SC seems less compatible with a clonal CD34+ SC proliferation and virus transfer but appears to indicate that also non-infected CD34⁺ SC are continuously recruited from progenitor cells and locally triggered to develop permissiveness to HHV-8 infection [21, 22]. Furthermore cells belonging to the non-cycling SC (Ki67-) population showed a clear increase during development from patch/plaque (median 13.5%) to nodular (median 40.3%) [21], also supporting the concept of continuous recruitment of CD34+ cells to the lesion. KS spindle-like cells have been shown to occur in cultures of peripheral blood of HIV infected patients with KS or at high risk for developing KS [43]. Furthermore recent studies show that endothelial cells or their precursors residing in donor kidneys may contribute to post-renal transplant KS as demonstrated by the finding that KS SC in the female recipient kidney had a male (donor) karyotype and that KS SC expressed the donor HLA antigen [44]. These findings seem to indicate that KS SC and/or their progenitors can be recruited during development of the KS lesion.

Characteristic spindle cells (SC) express various “mixed” (LEC and VEC) endothelial phenotypic cell markers possibly representing heterogeneous phenotypes of endothelial cells at different maturation stages. It has been recurrently debated whether SC are vascular (VEC) or lymphatic (LEC) in origin or derive from mesenchymal progenitor cells [45-47], although most studies by immunohistochemistry have revealed that SC express lymphatic markers,

such as D2-40 [48], LYVE-1 [45] and VEGFR-3 [46]. Also studies by gene expression microarray show that KS neoplastic cells are closely related to lymphatic endothelial cells (LEC) coexpressing some blood vascular endothelial cell (VEC) markers [49]. Furthermore HHV-8 can infect both LEC and VEC in vitro and infected LEC had a higher HHV-8 genome copy number than VEC[49]. In-vitro infection with HHV-8 of CD34+ human dermal microvascular endothelial cells (HDMEC) resulted in the upregulation of LEC markers such as LYVE-1 in the infected HDMEC [50].

In our study all LANA+ cells were LYVE-1+ (lymphatic endothelial markers) in early and late KS and HHV-8 infection (LANA) appeared better correlated to LYVE-1 than to CD34 expression [22]. LANA+/CD34- cells were more frequent in early as compared to late lesions and did not express a leucocytic phenotype (CD3, CD20, CD45, CD68) [21], but most expressed lymphatic endothelial (LEC) markers such as LYVE-1, VEGFR-3 and D2-40, suggesting that resident LECs represent an early target of primary HHV-8 infection [22]. This is also supported by other findings (Wang et al.)[49] that infected LECs have a higher HHV-8 genome copy number than VECs. Obviously a high viral copy number may result in an efficient maintenance and propagation of episomal HHV-8 DNA in dividing and migrating LECs. Furthermore in-vitro activation of VEGFR-3 by HHV-8 has been shown to increase endothelial cell migration and to enhance cell susceptibility to HHV-8 infection and transformation [40]. Hence, the activation of VEGFR-3 in LANA+/VEGFR-3+ SC observed during KS development will probably promote an increased endothelial cell migration (recruitment) and transformation to tumor SC including formation of pathological vascular slits.

Cell proliferation is relatively low in KS as shown by our previous studies on proliferation related protein Ki67 expression and by DNA flow cytometry [27]. The frequency of proliferating (Ki67+) cells usually decreased during development from early to late KS lesions, consistent with the notion that KS growth in the development from a reactive, early lesion to a nodular tumor depends not only on SC division but also decreased apoptosis [27] and progenitor recruitment [21, 22]. No significant difference in cell proliferation was observed between nodular AKS and EKS [21]. These findings could therefore indicate that the usually more aggressive growth of the AKS tumors may reflect a higher rate of SC progenitor recruitment compared to the more indolent EKS lesions.

Cytogenesis of KS

Reports on cytogenetic and molecular genetic changes in KS are few [51]. Studies from KS cell lines, KS Y-1 (AKS derived) and KS SLK (IKS derived) revealed loss of copies of chromosomes 14 and 21 and non-random translocations and deletions in the short arm of chromosome 3 at region 3p14. These KS cell lines also exhibit loss of heterozygosity of loci at region 3p14-ter. The chromosome 3 alterations observed were suggested to contribute to the neoplastic process in KS [52] but other cytogenetic studies on the KS-IMM cell line (IKS) [51] showed gains in 1q10→qter, 7p10→pter, 7q22→qter, 8p11→qter, 14pter→q22 but no changes in chromosome 3 [51]. These aberrations are compatible with the notion that initially KS may develop as a reactive polyclonal cell proliferation associated with chromosome instability, followed by acquisition of clonal chromosome changes in later stages [51].

The chromosomal instability suggested by the studies on cell lines may lead to cell apoptosis via the p53 pathway[53]. However, HHV-8 LANA binds to p53 repressing its ability to induce apoptosis [54]. Furthermore telomerase activity has been found to be upregulated in KS [55], which may immortalise the infected cell leading to increased tumor cell survival.

Previous, CGH studies of formalin fixed paraffin embedded KS biopsies revealed a recurrent gain at 11q13 [56], which also amplifies two known oncogenes, FGF4 and INT2, residing at 11q13 suggesting a possible role of HHV-8 as an integrating oncovirus that causes amplification and activation of genomic oncogenes in humans [56].

Loss of chromosome Y was observed in most AKS and EKS cases recently studied by us (57) and interestingly it was the only aberration observed in early KS. Late stage (nodular) KS had beside besides loss of chromosome Y, also recurrent deletions on chromosomes 16 and 17. Deletion of chromosome Y was also reported by previous studies on short term cultures of primary KS tumor cells and established KS cell lines [51]. EKS showed more chromosomal abnormalities than AKS (57), which might indicate that genomic instability could be a more important factor in the development of EKS than AKS. Most likely AKS development is also promoted by various cytokines and growth factors produced by the HIV infection and the dysregulated and compromised state of host immune response in HIV infection. Loss of the Y chromosome and encoded male specific minor histocompatibility antigens (HY antigen) has been shown to be linked to haematological relapse in acute lymphoblastic leukemia due to immune escape mechanisms [58]. The HY antigens are presented at the cell surface with the major histocompatibility complex (MHC) and together also processed intracellularly [59]. However, no studies have previously indicated a deficiency of HY antigen in KS tumors,

which the loss of Y chromosome from our studies suggests as of possible importance in KS pathogenesis.

HIV-Tat

The increased incidence of KS in patients with AIDS was shown to be particularly related to the effects of HIV-1 transactivating gene (Tat) protein which is taken up by cells and stimulates proliferation and inhibits apoptosis of the infected spindle cells. Apparently the Tat protein promotes AIDS KS by at least two distinct mechanisms. Thus, Tat competes with β -fibroblast growth factor (β -FGF) for heparin sulfate binding sites increasing the concentration of blood β -FGF, which is a potent angiogenic factor [60]. Furthermore, Tat has been shown to activate HHV-8 replication in BCBL-1 cells and PBMCs from patients with AIDS and PEL or KS [61] thus increasing viral load and expression of various viral genes with oncogenic potential including vGCR, vBCL2, and vIRF1. Thus, Tat promotes tumorigenesis of endothelial cells, both via stimulated synthesis of vascular endothelial growth factors, anti-apoptotic activity and HHV-8 replication. Notably, the functional activity of Tat protein in the pathogenesis of AKS clearly involves an intercellular signalling cascade which is inhibited by antibodies to HIV-Tat epitopes [62, 63]. This is corroborated by serology studies showing a deficient anti-Tat response in AKS patients compared to HIV-positive non-AKS subjects [62] and indicating the importance of functional Tat to promote AKS development and aggressiveness [64].

In summary the concept of oncogenesis related to infection is particularly well exemplified by the herpes virus HHV-8 and retrovirus HIV-1 associated Kaposi's sarcoma, which develops due to the effects of various host-cells and viral factors elicited during infection affecting cell proliferation, cell escape from apoptosis and dysregulation of host immune responses.

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Figure Legend

Fig 1. *HHV-8 gene expression(pathogenesis) during SC development and tumor growth.*

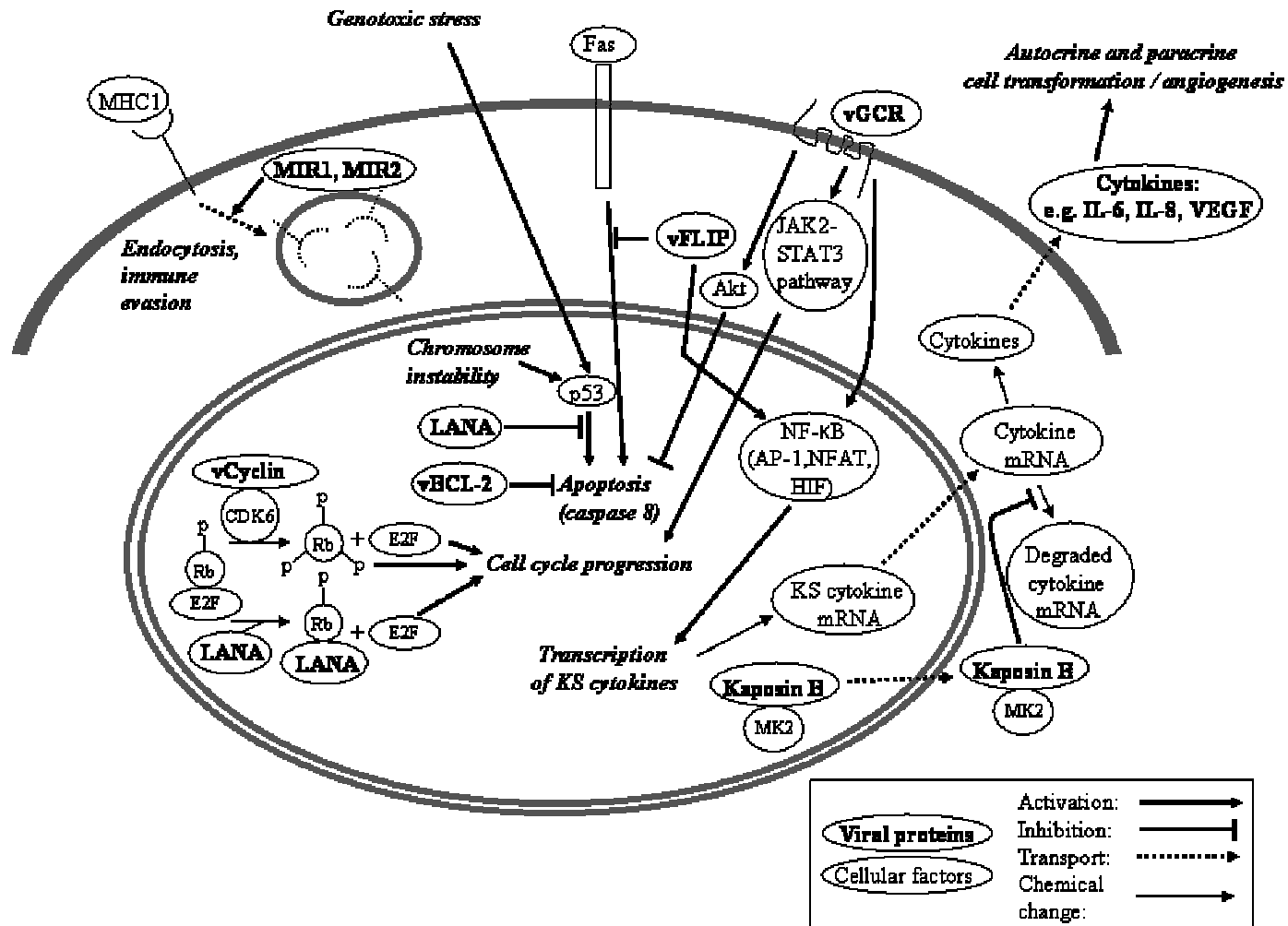


Fig 1