Chapter 15
Infection of KSHV and Interaction with HIV: The Bad Romance

Jie Qin and Chun Lu

Abstract Kaposi's sarcoma-associated herpesvirus (KSHV), namely, human herpesvirus 8 (HHV-8), is considered as the pathogen of Kaposi's sarcoma (KS), the most frequent cancer in untreated HIV-infected individuals. Patients infected with HIV have a much higher possibility developing KS than average individual. Researchers have found that HIV, which functions as a cofactor of KS, contributes a lot to the development of KS. In this article, we will give a brief introduction of KS and KSHV and how the interaction between KSHV and HIV contributes to the development of KS. Also we will take a glance at the development of treatment in KS, especially AIDS-KS.

Keywords Kaposi's sarcoma-associated herpesvirus (KSHV) • Human immunodeficiency virus (HIV) • Coinfection • HIV viral proteins • Treatment

15.1 Kaposi's Sarcoma-Associated Herpesvirus and Pathogenesis

15.1.1 Kaposi's Sarcoma and Kaposi's Sarcoma-Associated Herpesvirus

With complex histology feature, Kaposi's sarcoma (KS) shows abnormal vascular proliferation peculiarity. There are four types of KS, including classical KS, mainly affecting elderly men of Mediterranean or eastern European Jewish ancestry; AIDS-related KS, as its name shows, happens to AIDS patients; iatrogenic KS, usually

J. Qin • C. Lu (✉)
Key Laboratory of Pathogen Biology (Jiangsu Province), Nanjing Medical University, Nanjing, People's Republic of China

Department of Microbiology, Nanjing Medical University, Nanjing 210029, People's Republic of China
e-mail: clu@njmu.edu.cn

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happens to immunosuppressive patients after organ transplant; and African endemic KS, existing in parts of Central and Eastern Africa [1–4].

Investigation on KSHV seroprevalence shows that distribution of KSHV-positive individuals differs in regions and subpopulations. A report has been made that all forms of KS are more common in men than in woman, and further investigation showed that men from sub-Saharan Africa (50% KSHV prevalence) but not men from other district have a higher prevalence of KSHV than women [5, 6]. KSHV prevalence also shows distinct district differences. In endemic district, such as Uganda, KSHV prevalence of 50% has been reported, while in the USA, the report is 6% or even lowers [7–9]. Also in Xinjiang Uyghur Autonomous Region, China, a traditional endemic area, KSHV prevalence is much higher than other districts in China, with Han group showing a distinct lower rate [10]. Outside the endemic district, men who have sex with men (MSM) show a much higher KSHV prevalence than the average population. All around the world, KSHV prevalence are much high in MSM [11–14]. KSHV can also be found in saliva, and it is also reported as the highest shedding place; oral exposure to infectious saliva can be the transmission route of KSHV both sexually and nonsexually [15–17]. More researches have proved that in nonendemic districts, KS is more likely to happen to HIV-infected/AIDS population [18]. A recent study shows that HIV-1-infected children and adolescents in nonendemic districts have a higher possibility of KSHV seroprevalence [19].

Infectious saliva is the major route of KSHV transmission. However, increasing infection of KSHV among MSM strongly suggests that KSHV might transmit through sexual contact. More research has to be done to validate though [20].

Despite the fact that the discovery of KS is early in the late nineteenth century by Hungarian dermatologist Moritz Kaposi, it was not until the 1990s that KSHV, now considered as the pathogen of KS, was detected in KS tissues. In 1994, Chang and Moore identified KSHV genome in KS lesions [21]. They used representational difference analysis (a PCR-based technique) to identify and characterize alien DNA sequences in KS tissues. Sequences homologous to, but distinct from, capsid and tegument protein genes of the gammaherpesvirus *Saimiri* and Epstein-Barr virus were found in these tissues [21]. Now, 20 years has gone since this remarkable discovery. The characteristics of this virus have been mapped out by numerous scientists. Except KS, KSHV is also related to other two malignancies, primary effusion lymphoma (PEL) and multicentric Castleman disease (MCD) [21, 22].

15.1.2 KSHV Genome and Life Cycle

Soon after the discovery of KSHV, the genome of KSHV was mapped with cosmid and phage genomic libraries from the BC-1 cell line [23]. This group from New York found that the BC-1 KSHV genome consists of a 140.5-kb-long unique coding region flanked by multiple G+C-rich 801-bp terminal repeat sequences [23]. Now it has been found that KSHV encodes at least 86 open reading frames (ORFs), which

are expressed during distinct phases of KSHV infection [23]. It is believed that among these ORFs, 22 of them have the capacity of modulating immune response, such as K3, K5, K7, and K11.1 [24].

As a member of the γ-herpesvirus, KSHV has two distinct phases of infection as well, the latent and lytic phases [24]. After primary infection, both latent and lytic genes are expressed. Expression of lytic genes is shut down after a few rounds of replication to avoid immune surveillance, and latent infection of KSHV is established. During latency, KSHV expresses a few viral genes, ORF73 (latency-associated nuclear antigen 1 [LANA-1]), ORF72 (viral cyclin [vCyclin]), ORF71 (K13/vFLIP), and ORFK12 (kaposins A, B, and C), along with at least 12 distinct microRNAs [24, 25]. These all together facilitate the establishment of KSHV latency in hosts for a lifetime, survival against the host innate, and adaptive immune surveillance mechanisms, contributing to KSHV-related malignancies [24]. These genes and miRNAs expressed during latency also aid malignant transformation and oncogenesis by coping with several signaling pathways [26]. Among them, KSHV LANA directly deregulates signaling pathways such as MAPK, JAK/STAT, MEK/ERK, PI3K/AKT, Notch, and Wnt signaling to help establish latent infection [24, 27, 28].

Multiple chemicals, including tetracycline [29], are able to trigger KSHV reactivation. Once lytic replication is activated, immediate early (IE), early and late genes are expressed [30]. Production of lytic genes switches infected cell into intense viral replication, contributing to KSHV-induced tumorigenesis [24, 30]. These proteins encoded by KSHV lytic genes are also involved in modulating immune system or pathogenesis. For instance, K2-encoded vIL-6 can regulate B-cell proliferation by activating JAK/STAT, MAPK, and PI3K/Akt signaling pathways [31].

15.1.3 Noncoding RNA Encoded by KSHV

KSHV also expresses noncoding genes during latent or lytic phase. During lytic production, a 1.1-kb-long long noncoding RNA, which is now known as polyadenylated nuclear RNA (PAN RNA), is produced to facilitate KSHV lytic production [32]. Recent study also shows that this particular noncoding RNA encodes three peptides [33]. And with chromatin isolation by RNA purification coupled with next-generation sequencing (ChIRP-seq), PAN is found binding to KSHV genome to initiate lytic phase [33].

MiRNAs are expressed in latent cells, helping establish lifetime infection in host cells. MiRNAs are a group of small, about 22 nt in length, noncoding RNAs that are capable of regulating gene expression posttranscriptionally [34, 35]. The mechanism of how these small RNAs works has been studied since its discovery. It is believed that miRs can regulate gene expression through inhibiting transcription or destabilizing target genes by targeting complementary sequences in the 3′ untranslated regions (3′UTR) [34–37].

Discovery of KSHV miRNAs went through a history of a half decade. In 2005, Pfeffer et al., Cai et al., and Samols et al. identified 11 precursor-miRNAs (pre-miRNA) coded by KSHV by cDNA cloning strategies [38–40]. Later, with the help of a combined computational and microarray-based approach, Grundhoff et al. uncovered a different hairpin that leads to the 12th pre-miRNA, miRNA-K12, as well as most of the miRNAs discovered before [40]. With more digging, in 2010, different groups ascertained that there were at least 25 mature miRNAs deriving from those previously found pre-miRNAs [41]. No more miRs have been found ever since.

In 2013, a group in the USA found out that KSHV miRNAs are essential for tumorigenesis of KS. In this particular research, they found that deletion of KSHV miRs fails to transform, and instead it caused cell cycle arrest and apoptosis [26]. These results show that KSHV miRs are of great significance in the tumorigenesis of KS. And in this same research, NF-κB pathway is found to be the critical pathway targeted by KSHV miRs [26].

Moreover, these miRNAs are capable of regulating viral life cycle and gene expression, facilitating the tumorigenesis of KS. In a project done by Lu et al., they discovered that KSHV miR-K3 regulates viral latency by targeting nuclear factor I/B (NFIB), which indicates that KSHV miRNAs play a significant role in KSHV life cycle [42]. Also, miR-K3, miR-K4, miR-K7-5p, and miR-K9 have been reported to be related with the KSHV lytic switch protein (RTA)-regulated KSHV life cycle [42–45]. Moreover, a recent study showed that KSHV miRNA miR-K12-6-5p (miR-K6-5) can directly target and suppress a human gene, breakpoint cluster region (Bcr), resulting the activation of Rac1-mediated angiogenesis [46]. MiR-K1 also target IκBα, leading to NF-κB-dependent viral latency [47].

Researches on miRs are developing rapidly. A lot of the target genes or pathways regulated by miRs have been confirmed. For example, by inhibiting SH3BGR, miR-K6-3p activates STAT3 pathway to aid the malignancy of KS [48]; and by targeting GRK2, miR-K3 activates the CXCR2/AKT pathway, which influences the angiogenesis, migration and invasion of KSHV-infected primary human umbilical vein endothelial cells (HUVECs) [49]. A lot more targets of KSHV miRs have been confirmed, parts of the targets are shown in Table 15.1.

15.2 Interaction Between KSHV and HIV Viral Proteins

Although KSHV is the pathogen of KS, KSHV alone is not sufficient for the tumorigenesis of KS. HIV infection is thought as the cofactor in tumorigenesis of KS [50]. Epidemiology research on KSHV showed that KS is of higher possibility developing in AIDS patients [51, 52]. The HIV-KSHV interaction must have a place in KS.

HIV genome encodes 16 viral proteins, which all play essential roles in HIV life cycle. In the coinfected hosts, more cytokines are induced by HIV-1 effecting KSHV life cycle. Experiment done on BCBL-1 cells found that cytokines, like OSM,

Table 15.1 KSHV miRNAs and confirmed target genes

miRNA	Related pathways or genes	Ref.
miRNA-K12-1	Nuclear factor-κb (NF-κB)	[109, 110]
	Signal transducer and activator of transcription 3 (STAT3)	
	Casp3	
miRNA-K12-3	G protein-coupled receptor (GPCR) kinase 2	[49, 110, 111]
	Casp3	
	Nuclear factor I/B (NFIB)	
miRNA-K12-4	Casp3	[110]
miRNA-K12-5	Tumor suppressor protein tropomyosin 1 (TPM1)	[112]
miRNA-K12-6	Breakpoint cluster region (Bcr) protein	[46, 48]
	SH3 domain-binding glutamate-rich protein (SH3BGR)	
miRNA-K12-7	Replication and transcription activator (RTA)	[44]
miRNA-K12-9	Interleukin-1 receptor (IL-1R)-associated kinase 1 (IRAK1)	[113]
MiRNA-K12-11	SMAD5	[114]

HGF/SF, and IFN-γ, can induce KSHV lytic reactivation [50]. Same results are shown in other two PEL cell lines, BC-1 and BC-3 [50].

Multiple signaling pathways are involved in HIV-1 induced KSHV reactivation. HIV-1-infected BCBL-1 activated several pathways, including phosphatidylinositol 3-kinase/AKT (also called protein kinase B, PKB), mitogen-activated protein kinase (MAPK), and nuclear factor-κB (NF-κB) signaling pathways [53]. All these three pathways are involved in KS pathogenesis. In HIV-1-infected BCBL-1 cells, phosphorylation of PI3K and AKT is dramatically increased, and meanwhile the negative regulator of PI3K and PTEN is decreased, all leading to the activation of the PI3K/AKT pathway [53]. Moreover, HIV-1-infected BCBL-1 cells showed increased expression of Ras and phosphorylation of c-Raf, MEK1/2, and extracellular signaling-regulated kinase (ERK), which all represent their activation [53]. Furthermore, activation of the Ras/c-Raf/MEK1/2 MAPK pathway leads to the activation of KSHV lytic production [53]. However, the role of NF-κB in the reactivation of KSHV remains a controversy.

15.2.1 HIV-1 Tat and Its Function in the Oncogenesis of KS

The HIV-1 Tat is a polypeptide with a length of 86–104 amino acids (aa) [54]. With its ability to transactivation, HIV-1 Tat is vital for HIV replication [54]. Extracellular Tat is capable of entering uninfected cells and transactivate endogenous genes, such as tumor necrosis factor, interleukin-2 (IL-2), and IL-6 [55]. Tat is positively charged, and with this feature, it is able to bind to negatively charged molecules, such as VEGFR-2, which significantly promotes angiogenesis in vivo [54]. With

this effect, there is high possibility that Tat contributes to KSHV-inducing abnormal angiogenesis in KS formation.

Researches confirmed that Tat, regulatory protein encoded by HIV, is involved in several activities of KSHV. It has been proved that Tat, as a cofactor in pathogenesis of AIDS-KS, is a growth factor for KS spindle cells [56, 57]. Transgenic expression of Tat in mice helps in the formation of KS-like lesions [58]. Researches so far have found that HIV Tat can affect KSHV life cycle and facilitate AIDS-KS by inducing cellular proliferation and pro-inflammatory genes. In 2007, our group found that, by inducing human interleukin-6 (huIL-6) and its receptor (huIL-6Ra), Tat enhances KSHV lytic replication through modulation of the JAK/STAT pathway [59].

Far in the late 1990s, it is demonstrated that, for Tat being capable of inducing pro-inflammatory and proliferative genes in KS, it might contribute to the pathogen of KS [60]. Tat enhances the expression of IL-6, MCP-1, ICAM-1, and VCAM-1 in cultured KS cells [60]. Among these cytokines, IL-6 is a cytokine that activates leukocytes and induces the proliferation of KS cells [60]. The expression of MCP-1 and other cellular adhesion molecules could in return promote the expression of IL-6 [60].

In cooperation with a 13-amino-acid peptide corresponding to the basic region of Tat, HIV-1 Tat enhances KSHV infectivity by aiding KSHV entering into endothelial cells and other cells [61]. This might be the reason AIDS-KS is far more aggressive than KS in other immunodeficiency or immunocompromised states. In the pathogenesis of KS, HIV-1 Tat may cooperate with KSHV-encoded genes to facilitate KS tumorigenesis. Research found that HIV-1 Tat may enhance KSHV kaposin A-mediated tumorigenesis in vitro and in vivo through several signaling pathways, such as MEK/ERK, STAT3, and PI3K/Akt signals [58]. However, it is not only that kaposin A-mediated tumorigenesis is enhanced by Tat but also vIL-6. Through activating PI3K and AKT and inactivating PTEN and GSK-3β, Tat significantly promotes vIL-6-induced angiogenesis and tumorigenesis of fibroblasts and human endothelial cells in a chicken chorioallantoic membrane (CAM) model [62].

Most of the cells in KS are under latent infection; however, a few KSHV-infected cells are activated and express lytic genes, such as Orf-K1 and Orf-K2 [63]. Soluble Tat or ectopic expression of Tat enhanced K1-induced cell proliferation and angiogenesis in vitro and in vivo [63]. In synergy with K1, Tat induces the expression of miR-891a-5p of host cells, which activates NF-κB by targeting IκBα 3′ untranslated region [63]. Activation of NF-κB in turn contributes to the malignancy of KS.

Moreover, ectopic expression of HIV-1 Tat promotes HSV-2-induced KSHV reactivation, resulting in KSHV going into lytic phase [64].

15.2.2 HIV-1 Nef and Its Role in Tumorigenesis of KS

Nef, expressed during the early stage of infection, is encoded by the *nef* gene, which only exists in primate lentiviruses [65]. In 1991, Kestler et al. infected Rhesus macaques with a mutated strain of $SIVmac_{239}$ lacking the Nef ORF, which proved

that the *nef* gene is vital in maintaining high viral load and viral infection [66]. Nef is structurally multifunction. Far in the 1990s, multiple groups confirmed that in HIV-1-infected cells, Nef assembles on the cell surface or in cytoplasm [67, 68]. Myristoylation of Nef and basic amino acids on its N-terminal helps the interaction between Nef and membrane [68, 69], which facilities its coping with host contents and helps the replication of HIV-1 [70]. Different groups confirmed that Nef is also able to enhance the infectivity of HIV-1 [70, 71]. Recent study shows that Nef is also involved in the localization of Gag, resulting in transferring viruses cell to cell [72]. With its ability to interact with multiple host factors, Nef displays remarkable ability in connecting with the cellular vesicular trafficking machinery and to perturb cell signaling [65].

Not only is HIV-1 Nef of great importance in HIV-1 infection, but also it plays significant roles in the oncogenesis of KSHV. Based on the fact that Nef localizes in the pulmonary arterial endothelial cells of AIDS patients, our group validated that in cooperation with KSHV viral interleukin-6 (vIL-6), HIV-1 Nef facilitates angiogenesis and oncogenesis of KSHV by manipulating AKT signaling pathway [73]. The experiment in vivo shows Nef boosts vIL-6-induced angiogenesis and tumorigenesis [73]. In this particular research, we found that exogenous Nef is able to penetrate endothelial cells, without impacting the apoptosis of endothelial cells [73]. That corresponds with Nef being able to get to cell membrane.

Despite vIL-6, HIV-1 Nef works in synergy with KSHV K1 to promote cell proliferation and tubulogenesis of human umbilical vessel endothelial cells (HUVEC) [74]. HIV-1 and KSHV K1 together induce cellular miR-718, which in turn regulates the PTEN/AKT/mTOR signaling pathway [74].

Moreover, Nef is capable of regulating KSHV life cycle. Our recent investigation shows that soluble and ectopic Nef can suppress KSHV lytic replication to promote latency in PEL cells [75]. Mechanism study revealed that cellular miR-1258 enhances Nef inhibition of KSHV reactivation [75].

Besides Tat and Nef, HIV-1 viral protein R (Vpr) is another viral protein that is involved in regulating KSHV life cycle. Researchers found that Vpr is able to activate KSHV transcription [76]. And with its ability of internalizing into PEL cells, Vpr can activate NF-κB signaling pathway, and cellular miR-942-5p directly target inhibitor of NF-κB, revealing the role of NF-κB in balancing KSHV latency and lytic production [77].

15.2.3 KSHV Affects HIV

HIV influences KSHV in multiple ways and plays important roles in the oncogenesis of KS; KSHV in turn influences host cell susceptibility of HIV-1 and replication in few ways as well [78]. The receptor for KSHV, DC-SIGN, is expressed on activated macrophages, B cells, and monocyte-derived dendritic cells (MDDCs). Also, isoform of DC-SIGN, DC-SIGNR, is also expressed on endothelial cells [79].

Among them, dendritic cells are of great significance in HIV-1 infection, which indicates the relationship of KSHV and HIV-1 in their coinfection.

KSHV plays a role in HIV viral transportation. Research found that dendritic cells stimulated by KSHV capture more HIV viral particles and enhance HIV-1 transport to CD4+ T cells, which is a key route of HIV-1 transfer between cells [80].

KSHV is also involved in regulating HIV-1 life cycle. KSHV ORF50 (encodes RTA) is an important gene in KSHV reactivation [81]. In KSHV and HIV coinfection case, KSHV ORF50 increases cell susceptibility of HIV-1 infection in vitro and is capable of transactivating the HIV-1 LTR in synergy with HIV-1 tat gene [81, 82]. In susceptible cell, like T cells and B cells, the expression of ORF50 activates HIV-1 replication, and in unsusceptible cells, HIV-1 alone is not able to launch reactivation, while transformed with ORF50, HIV-1 infection is more persist in parent cell and leads to low level of HIV-1 virus production, infecting susceptible cell by direct contact [83]. Meanwhile, KSHV ORF57 is found being able to activate HIV-1 replication by regulating ORF50 or other unidentified mechanism [82].

Despite OFR50, researchers found that KSHV-encoded ORF45 was the most robust in mediating transcriptional activation of HIV-1 TLR via the cellular p90 ribosomal S6 kinase (RSK2) as well [83].

In addition, KSHV-encoded viral FLIP (Fas-associated death domain-like IL-1 beta-converting enzyme inhibitory protein) K13 can activate the HIV-1 LTR in cooperation with HIV Tat [84]. The activation is done via K13 activating NF-κB pathway [84].

Moreover, KSHV latency-associated nuclear antigen (LANA) is constantly expressed in KSHV-infected cells. Research found that by functioning as a regulator of transcription, LANA is able to transactivate HIV-1 LTR in multiple cell lines, including human B-cell line BJAB, human monocytic cell line U937, and the human embryonic kidney fibroblast cell line 293 T [84]. And HIV-encoded Tat protein is in cooperation with LANA in the reactivation [84].

15.3 Effect of Antiviral Treatment on KS Development and New Treatment of KS

After the epidemic of HIV infection and outbreak of AIDS, till now, multiple anti-HIV drugs have been approved by US Food and Drug Administration (FDA). And in HIV-infected individuals, antiretroviral treatment (ART) is sufficient to prevent transmission [85–87]. At the same time, HAART has significantly reduced KS incidence in HIV-positive patients, while in Africa, where antiretroviral drugs are not easily accessible, KS remains a problem for HIV-infected patients [88, 89]. Effects of HAART on AIDS-KS are diverse, including inhibition of HIV replication, improved immune response, or direct inhibition of HIV-1 Tat [90]. However, no scant evidence or clinical evidence shows that HAART alone is sufficient to treat KS [89, 90]. KS several treatment methods have development in treating KS, while no standard methods have been made.

HAART, in combination with systemic and local therapy, is efficient in controlling KS, resulting in regression of KS both in size and number of KS lesions [88, 91, 92]. Such regimens include cytotoxic chemotherapy and protease inhibitor [90]. A trail involving chemotherapy and HAART elucidated that a combination of HAART and chemotherapy achieved higher overall KS response, resulting in higher overall survival and improved quality of life [93]. Chemotherapy is strongly recommended in treating KS, especially KS with pulmonary involvement [20]. HAART mainly controls HIV, while chemotherapy is specific to KS.

Together with HAART, FDA-approved chemotherapeutic drugs including pegylated liposomal doxorubicin (PLD), liposomal daunorubicin, and taxane paclitaxel are proved impactful in treating KS. PLD plus HAART showed better KS response after 48-week treatment than HAART alone, and it shows equal efficiency in advanced KS [20, 94, 95]. Later year, in 2005, researcher found that this combination can induce effective tumor remission and recovery of CD4+ cells [96]. The comparison between paclitaxel and PLD showed similar response toward KS (a rate of 50–60%), with paclitaxel showing hematologic toxicity and more alopecia and sensory neuropathy [97]. And liposomal daunorubicin was approved by US FDA as the first-line treatment of KS [98]. And KS patients benefit from higher cumulative chemotherapeutic doses without significant cardiotoxicity [99]. However, HAART in combination with chemotherapy is not as effective as expected. Still 51% of the patients have persistent KS 36 months after diagnosis.

New drugs targeting KSHV regulated pathways or factors are developed during recent decades. Rapamycin, an mTOR signaling pathway inhibitor, is proved effective in transplant-related KS, and in AIDS-KS, its effect still needs further investigation [100, 101]. And also there is a report on classic-KS regression after treatment with rapamycin [102]. Drugs or immune modulators like interferon-a, interleukin-12, thalidomide, and lenalidomide are effective either alone or in combination with other treatment [103–106]. Other drugs targeting KSHV-encoded genes regulated signaling or KSHV-induced angiogenesis; apoptosis is also under investigation [90].

15.4 Remarks and Perspectives

In this article, we made a discussion on KSHV and the heated topic of KSHV miRNAs during the last few years. These products of KSHV latency are of great significance in the angiogenesis, migration, and invasion of KS. This leads us one more step closer to the myths of KSHV and KS. However, cell origin of KS is still controversial and haunting around. The establishment of KSHV-infected MSCs is the first step in searching the secret behind KS [107, 108]. Besides that, the network of interaction between KSHV miRNAs and its target genes deserves more digging to clarify the underlying secrets of KSHV miRNAs in the tumorigenesis of KS. Researches on HIV and KSHV coinfection now mainly focus on the HAART treatment. Drugs and methods in treating AIDS-KS have been found and proved

effective, saving thousands of lives. However, works on prevention of KS is still slow. No vaccines or other drugs have been found or developed in preventing KSHV infection or KS development. And works on non-AIDS-related KS still need more attention.

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