Review

Molecular biology and pathogenesis of Kaposi sarcoma-associated herpesvirus

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Abstract

Kaposi sarcoma (KS)-associated herpesvirus (KSHV) is the most recently discovered human oncogenic herpesvirus. The virus is associated with KS lesions and other human malignancies, including pleural effusion lymphomas and multicentric castleman's disease. The sequence of the viral genome demonstrated that it belongs to the gammaherpesvirus family similar to the Epstein–Barr virus, the only other known human herpesvirus associated with human cancers. Molecular studies have identified a number of viral genes involved in regulation of cell proliferation, gene regulation, chromatin remodeling and apoptosis. KSHV transforms human endothelial cells in vitro with low efficiency and expresses a repertoire of latent genes involved in the establishment of latency. One of these latent proteins, the latency-associated nuclear antigen (LANA) is required for episomal maintenance and tethers the viral genome to the host chromatin. LANA has now been shown to be a multifunctional protein involved in numerous cellular functions including binding to the retinoblastoma protein and p53, regulating cell proliferation and apoptosis.

1. Kaposi sarcoma (KS)-associated herpesvirus (KSHV) infection and human malignancies

Moritz Kaposi, a Hungarian dermatologist working in Vienna, first described KS lesions in 1872. He published a case report of five men with 'idiopathic multiple pigmented sarcoma of the skin' including a patient who developed visceral disease in the lung and gastrointestinal tract [1]. After two decades this idiopathic multiple pigmented sarcoma of the skin was designated KS at the suggestion of another prominent dermatologist, Kobner, and is now referred to as classic KS [1]. Prior to the acquired immunodeficiency syndrome (AIDS) epidemic, KS was rarely seen in North America and Northern Europe. However, the AIDS epidemic triggered KS to become the most common AIDS-associated cancer and represents one of the major contributors to morbidity and mortality in AIDS patients [2]. Additionally, HIV seronegative, homosexual men are potentially at higher risk for developing KS compared to the individuals in countries where the rates of KS are higher [3]. KS is also seen as a post-transplant neoplasm in the general patient population. These KS lesions regress when immunosuppressive therapies are withdrawn from transplant patients, suggesting modulation by the host immune system [4].

KSHV is the most recently discovered human gammaherpesvirus with tropism primarily for endothelial cells and B-lymphocytes, but can also infect other cell types with limited efficiency. It is the eighth human herpesvirus isolated to date and is thus also named human herpesvirus 8 (HHV8) [1,5]. This virus was initially identified from KS tissue but was later also found to be associated with pleural effusion lymphomas (PELs) or body cavity-based lymphomas (BCBLs) [5].

A preponderance of data strongly suggest that KSHV is likely to be the etiologic agent of KS and may also be a critical player in the development of other lymphoproliferative disorders including PELs and multicentric castleman's disease (MCD) [6–8]. Cell lines have been estab-

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lished from PELs, which contain the KSHV genome. Some of these cell lines are co-infected with KSHV as well as Epstein–Barr virus (EBV), another member of the gammaherpesvirus family shown to be associated with lymphomas and lymphoproliferative diseases (LPDs) in humans [8]. These lymphoblastoid cell lines co-infected with both KSHV and EBV or singly infected with KSHV were generated from lymphoma samples obtained from AIDS patients [7,8]. The major diseases associated with KSHV infection are discussed briefly.

1.1. Kaposi sarcoma (KS)

KS is an unusual multifocal neoplasm characterized by dark purple lesions, which differs from most other common tumors in that the lesions contain multiple cell types (polyclonal), with the dominant cell being the spindle cell, derived from endothelial origins [9] (Fig. 1A). In addition, the KS lesions contain numerous infiltrating inflammatory cells as well as a profusion of neovascular elements [10]. KS is characterized as a slow tumor, which resembles a human tumor with low malignant potential in immunocompetent patients [3]. This malignancy is more widespread in individuals who are immunocompromised and in these cases it can become detrimental to the patient and even fatal. It should be noted that in cases where the immune competence was restored this has led to the complete remission of the disease state, which is quite distinct from other more aggressive tumors [9,11].

Association of KSHV with PELs/BCBLs has also been documented and a number of cloned cell lines, which include BC-1 and BC-2, were shown to be co-infected with EBV [12]. However, several PEL cell lines including BC-3 and BCBL1 from HIV positive patients have also been described without detectable levels of co-infection with EBV [8,13]. Analyses of these cell lines show that they have clonal immunoglobulin heavy chain rearrangements suggestive of a B-cell origin. KSHV can therefore infect human B-cell lines and may be involved in driving these PELs in HIV positive AIDS patients. KSHV has also been shown to infect and replicate in other cell lines, albeit less efficiently than seen in the BCBL cell lines [14,15].

In the western population, KS is found most commonly in HIV positive patients. However, immunosuppression is not the only condition in which KS was detected in humans. There are four distinct clinical variants of the KS which is characterized based on the extent of immunosuppression and severity of infection. These include classic KS, which predominantly affects elderly men of Mediterranean and eastern European decent and is mostly indolent; endemic KS, which is prevalent in equatorial, eastern and southern Africa and is a clinically more aggressive form than classic KS [16]; iatrogenic or post-transplant KS, which develops in patients undergoing immunosuppressive therapy to prevent graft rejection after organ transplantation [17]; and AIDS-associated KS, which is the most aggressive form of the disease and is most commonly seen in gay and bisexual men suggesting that transmission is likely through high risk sexual routes [18].

1.2. Primary effusion lymphoma (PEL)

PEL, or BCBL, is a rare, rapidly fatal, non-Hodgkin's malignancy associated with KSHV infection. It is generally present as a pleural or pericardial effusion without a detectable mass or peripheral lymphoadenopathy [7] (Fig. 1B). Alternatively, PEL can present as a solid mass in the lymph nodes, lungs or the gastrointestinal tract [7]. PEL occurs predominantly in HIV seropositive individuals in advanced stages of immunosuppression and is also seen in HIV seronegative patients. Moreover, PEL cells are frequently found to be co-infected with EBV and KSHV, although EBV negative and KSHV positive PEL has also been described [7]. Most PELs are thought to originate from post-germinal center B-cells due to the presence of hypermutated immunoglobulin genes and markers of late stage B-cell differentiation, and are considered to be clonal due to the presence of clonal immunoglobulin gene rearrangement and monoclonal terminal repeats (TRs) [7]. Southern blot analysis of PEL cells shows that KSHV genome is maintained at a high copy number (50–150 per cell), which is substantially higher than that observed in KS-infected spindle cells [7].
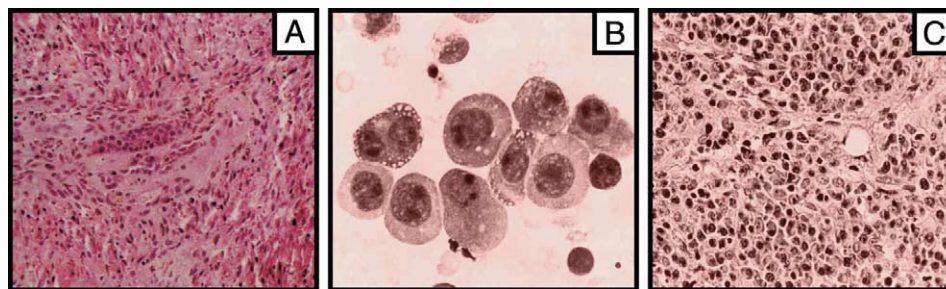

Fig. 1. Histopathology of KS, PELs and MCDs. A: Histological section of nodular tumor stage KS lesion stained with hematoxylin and eosin showing infiltrating spindle cells and disorganized vascular lumen filled with red blood cells. B: Hematoxylin and eosin-stained PEL cells from needle aspirate showing large plasmacytoid tumor cells. C: Hematoxylin and eosin-stained section of HIV-associated MCDs showing characteristic histopathology including plasmacytoid domination of node architecture.

1.3. Multicentric castleman's disease (MCD)

Castleman's disease is an unusual lymphoproliferative disorder thought to be mediated by interleukin (IL)-6 overexpression [19]. KSHV can be detected in most HIV seropositive cases of MCD as well as in approximately 40% of HIV seronegative MCD cases. KSHV positive MCD cases are now recognized as a distinct subset of MCD, named plasmablastic MCD, which contains large plasmablastic cells (Fig. 1C) harboring KSHV [20]. Unlike PEL cells, co-infection by EBV has not been detected in MCD plasmablasts.

2. Epidemiology of HHV8 infection

A number of proteins encoded by KSHV open reading frames (ORFs) during latency and lytic life cycle of the virus were initially characterized as a result of serological studies of patients with KS. These studies, based on immunofluorescence, Western blot and enzyme-linked immunosorbent assays to detect antibodies against latent and lytic genes, have demonstrated that KSHV is not ubiquitous throughout the general population as are the majority of other human gammaherpesvirus, including EBV. The seroprevalence to KSHV tends to be lowest in Asia, with 0.2% of blood donors in Japan testing positive, compared to 1–3% in the USA. However, this percentage is significantly higher (28%) in certain regions of Italy [21,22]. In areas highly endemic to KS, the range of seroprevalence of KSHV has been reported in some cases to be greater than 50%, which may account for the high general risk of KS in these areas. Indeed, KS is one of the most common cancers seen in the general population in regions of central Africa including Uganda and Kenya [16].

Studies have shown that viremia is not necessarily an essential component of KSHV infection, as only about 50% of patients with KS have detectable KSHV DNA in their peripheral blood mononuclear cells (PBMCs) as determined by PCR analysis [23]. Serological detection of specific antibodies to KSHV-encoded proteins is often life-long after infection and is more sensitive than PCR. In addition proteins expressed during latency and lytic infections can be useful antigens for the sensitive detection

by serum antibodies from KS patients. The minor capsid protein encoded by ORF65, and latency-associated nuclear antigen (LANA) encoded by ORF73 expressed during latent infection, are the two critical antigens used for the serologic detection of KSHV in infected patients. Serological detection of these proteins confirms that KSHV is present in association with KS and is detectable in 70–100% of patients with the disease [24,25].

3. Molecular detection and identification of KSHV

KSHV was discovered by the representational subtractive hybridization technique, which yielded two DNA fragments specific for the KS lesions. The nucleotide sequence of these bands showed similarity to ORFs of the gammaherpesvirus and most closely resembled *Herpesvirus saimiri* (HVS), a member of the Rhadinovirus genus or the gamma-2 lineage of the gammaherpesvirus subfamily, known to be associated with proliferative disease and cancers in human [26] (Fig. 2). The natural host of HVS is the squirrel monkey in which there is no associated LPD in vivo, however, HVS has the exquisite ability to growth transform human T-lymphocytes in vitro [27]. A recent discovery in Rhesus monkey identified the Rhesus Rhadinovirus, which has significant collinear homology and closer identity to KSHV than HVS [28]. In PBMCs, KSHV DNA can also be detected by PCR in approximately 50% of KS patients. Furthermore, KSHV detection in PBMCs of HIV seropositive individuals can be a predictor for the development of KS [23]. Interestingly, one group reported the frequent detection of KSHV in semen from healthy Italians, however this was not detectable in semen from donors in North America and the UK [27].

4. Molecular biology of KSHV

KSHV is a member of the gammaherpesvirus subfamily, Rhadinovirus genera, which share collinear genomic organization with each other [26]. Complete genome sequences of two KSHV isolates have now been determined, one from a PEL cell line and the other from a KS specimen, both revealing the characteristic collinear genomic organi-
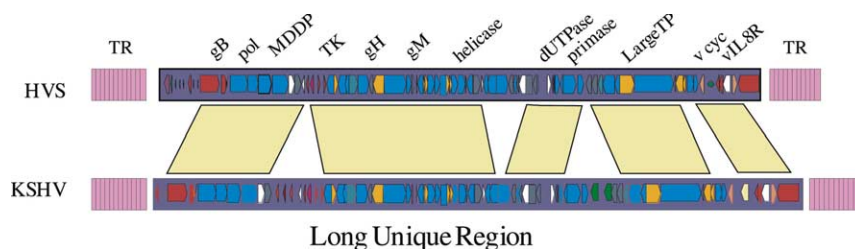

Fig. 2. A schematic showing the organization of the primate Rhadinovirus HVS and human virus HHV8. Note the long unique region and the TR elements flanking the long unique region. The collinear organization of these viruses shows blocks of genes arranged in a similar orientation with high homology to each other in structure and function.

zation of the Rhadinoviruses [26]. The KSHV is a double-stranded DNA virus of size approximately 165 kbp with a central low GC-content region called L DNA flanked by a highly repetitive sequence of high GC content, called H DNA. The L DNA region of the KSHV genome, spanning approximately 140 kbp, has at least 81 ORFs, including 66 with homology to HVS ORFs [26], and five internal repeat regions present in the long unique region (Fig. 2). H DNA is the TR elements of the KSHV genome, flanking the L-DNA region, and is approximately 85% GC content with 801 bp of reiterated DNA sequence [26].

A characteristic of KSHV and other gammaherpesvirus is the similarity of a large number of the ORFs to known cellular genes suggesting that some of these genes may be pirated from the host chromosomes during the process of viral evolution. Some of these genes are involved in down modulating the immune response, evading cellular systems important for targeting infected cells, nucleotide biosynthesis and for cell growth and differentiation [26,29]. They include the Bcl-2, IL-8R, and MIP-Iα, vIL-6, DHFR and the D type viral cyclin [26], whose functions are usually similar to that of their cellular homologs (Table 1). The degree of conservation of specific cellular homologs varies within members of the family suggesting that the individual members of the gammaherpesvirus family have evolved different strategies for bypassing the host immune response.

5. Latent infection

KSHV, like the other members of the gammaherpesvirus subfamily, latently infects predominantly B-cells and endothelial cells. Infected cells retain the virus from one generation to the next and can also growth transform the infected cells [3]. During latent infection the virus exists as a multicopy circular episomal DNA in the nucleus expressing a small subset of viral genes [6]. One of these proteins, which is consistently shown by in situ hybridization and immunohistochemical analysis, to be highly expressed in all forms of KS-associated malignancies, is LANA [30]. LANA is a 222–232-kDa nuclear protein expressed from ORF73 as a polycistronic mRNA along with the viral cyclin encoded by ORF72 and the viral Fas associated death domain IL-1B converting enzyme inhibi-

tory protein encoded by ORF71 [31]. Studies so far have indicated that LANA is important for maintenance of viral episomal DNA during latent infection and modulates viral and cellular gene expression. LANA was shown to tether the viral episomes to host chromosomes and binds to specific sites within and in close proximity to the TR element, thereby contributing to the stable maintenance of the viral episomes in successive daughter cells [32,33]. Therefore, LANA is a multifunctional protein essential for maintenance and possible segregation of the KSHV genome during cell division by tethering the viral genome to the host chromosome. LANA is also important for regulating gene expression in KSHV-infected cells.

5.1. Role of LANA in maintenance of KSHV

LANA is a large nuclear antigen detected in the majority of KS lesions as well as in cell lines derived from BCBLs [28]. Indirect immunofluorescence studies of BCBL cell lines with serum from KS patients revealed a characteristic punctate pattern of nuclear immunofluorescence due to the presence of LANA, which is important for maintenance of the KSHV episome in these BCBLs [34,35]. Studies using fluorescent in situ hybridization demonstrated that LANA associates with the KSHV genome in infected cells and that the KSHV episomes localize to host metaphase chromosomes in a similar punctate pattern [34,35]. Direct binding studies with KSHV DNA fragments indicated that LANA displays preferential binding to different regions of the KSHV genome with at least three regions showing relatively strong binding. A region at the left end of the KSHV genome, containing TR elements, persisted in cells stably expressing LANA in *trans* suggesting that this region contained the *cis*-acting DNA element [35–38]. However, this does not exclude other regions of the genome as alternate elements capable of functioning as origins of replication during persistent infection. The major LANA binding cognate sequence (minimum of 13 bp) has now been mapped within the TR, which binds to the carboxy 200 amino acids of LANA [36,37,39]. Additionally, LANA was shown to bind to histone H1, but not to core histones, and tethers the viral episomes to the host chromatin [35] (Fig. 3). A deletion in the chromosome binding site in the amino-terminal region of LANA (5–22 amino acids) abolishes the episomal maintenance

Table 1
Major KSHV-encoded cellular homologs [26]

| Protein product	ORF	Function	Expression
vIL-6	K2	Activates gp130 independently of IL-6R. Autocrine growth factor in PELs. Angiogenic	Lytic replication by 10 h
vIRFs			
vIRF-1 and 2	K9 and K11	Blocks IFN- and IRF-mediated transcriptional activation	Lytic replication by 48 h
vMIP-I	K6	CCR8 agonist. Angiogenic	Lytic replication by 10 h
vBcl-2	ORF16	Inhibits bax-mediated and virally induced apoptosis	Lytic replication by 10 h
v-Cyclin	ORF72	Constitutively activates CDK6	Latent
vGPCR	ORF74	Constitutively actives GPCR. Binds IL-8. Oncogenic. Angiogenic?	Lytic replication by 10 h
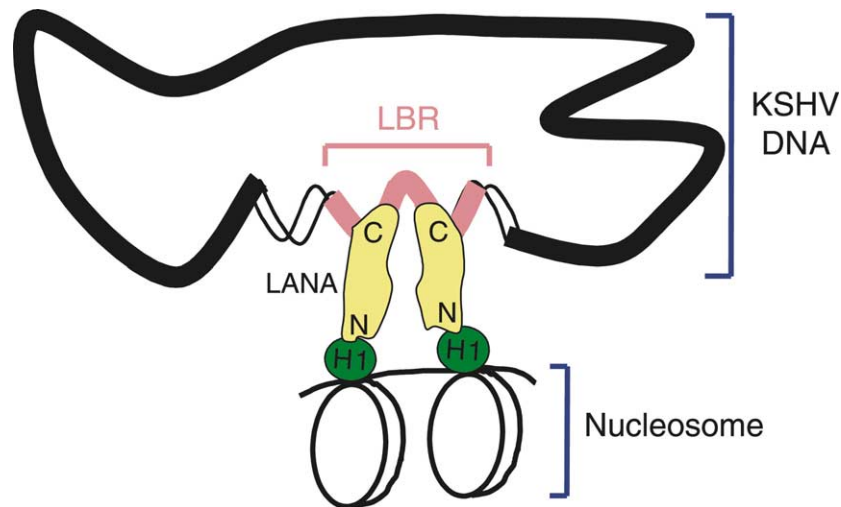
Fig. 3. A model showing the proposed mechanism of KSHV episome tethering to the host chromosome. The carboxy-terminus of LANA binds to a specific *cis*-acting DNA element (13 bp) in the TR of KSHV genome and simultaneously chromosome binding sequence in the amino-terminus of LANA binds to chromosomal linker protein histone H1. Physical anchorage of viral episome with the chromosomes ensures the viral persistence in the daughter cells after successive rounds of cell division.

which is restored by replacing the mutation with histone H1 protein [40]. These data indicate that LANA possesses a functional role similar to the EBV-encoded EBNA1 (EBV nuclear antigen 1) protein in maintenance of the EBV genome in viral-infected cells [35].

5.2. Role of LANA in regulation of transcription

The secondary structure of LANA suggests that there are potential sites for interactions with other cellular factors involved in transcription [35]. Its amino acid sequence indicates that it has an acidic-rich, a proline-rich and a glutamine-rich domain, a zinc finger DNA binding domain, a leucine zipper and a potential nuclear localization signal [26]. LANA binds a number of cellular proteins involved in transcriptional regulation such as CBP, RING3, activating transcription factor-4/cyclic AMP response element binding protein-2 and mSin3A [41,42]. There is evidence suggesting that LANA is involved in transcription repression through interaction with p53 and down modulating p53-mediated activation of its responsive promoters [43]. Additionally, LANA can repress transcription when fused to the GAL4 DNA binding domain, tested on a GAL4 responsive promoter [43]. LANA has also been shown to transactivate promoters regulated by E2F and associates with hypophosphorylated retinoblastoma protein (pRb) in transiently transfected cell lines [44]. LANA activates the human telomerase reverse transcriptase promoter as well as the HIV LTR cooperating with the Tat protein expressed by HIV-1 [45,46]. In addition, LANA activates the EBV LMP1 promoter suggesting a functional role in regulating EBV latent gene expression in co-infected BCBL cells including the upregulation of the EBV oncogene, LMP1 [47]. Therefore, LANA is a multifunctional protein involved in modulating activation and repression of transcription. These activities are important in regulation of cell proliferation and apoptosis in KSHV-infected cells (Fig. 4).

6. Lytic infection cycle

The majority of the KSHV genome remains silent during latent infection. The program of gene expression during lytic replication was revealed by DNA array expression profiling of PEL cell lines [48]. The first groups of genes expressed after the induction of lytic replication are typically regulators of gene expression including the immediate early transactivators ORF50 (Rta or Lyta), K8 (Zta or K-bZIP) and ORF57 (post-transcriptional regulator of gene expression). This is followed by the expression of sets of genes, involved in replication of the viral DNA including the DNA polymerase and its processivity factor [49]. The structural genes and those involved in virus expression and maturation are expressed later, generally after 24 h post-infection [49].

Studies have identified an ORF50 similar to EBV immediate early transactivator, which functions as an immediate early transactivator of KSHV lytic replication. It was demonstrated that this protein is capable of stimulating KSHV lytic replication in vitro similar to the Zta and Rta proteins of EBV [8].

7. Viral-encoded cellular homologs and their role in cell growth

Large DNA viruses like herpesviruses and poxviruses have many genes encoding cellular homologs. This is believed to be due the incorporation of host cell genes into
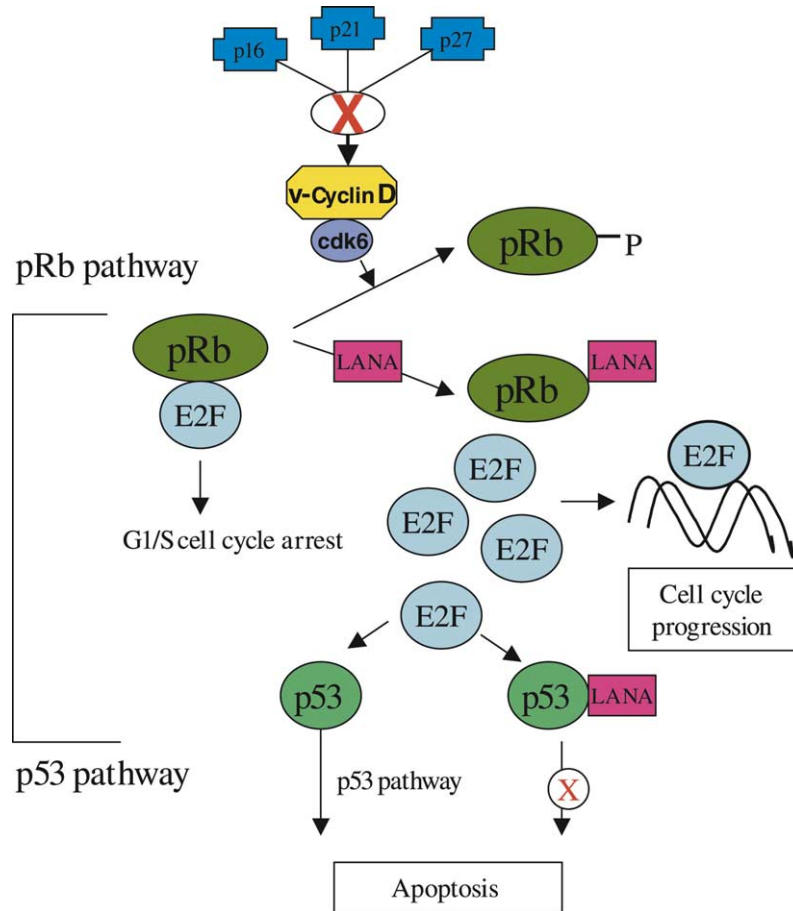
Fig. 4. A schematic model showing the role of LANA and v-cyclin in cell cycle progression. In normal uninfected cells G1/S progression is negatively regulated by binding of pRb to E2F transcription factor. In infected cells, LANA competes with E2F for binding of hypophosphorylated pRb thus freeing E2F to activate the transcription of genes involved in cell cycle progression. LANA also binds p53 and blocks the p53-mediated apoptosis [43].

the viral genome during years of evolution. KSHV encodes homologs of human cyclin D (vCYC) [50], G protein-coupled receptor (vGPCR) [29], chemokine homologs (vMIPs) [51], homolog of IL-6 [23], protein with similarity to interferon (IFN) regulatory factor (vIRF) [52] and a bcl-2 homolog [53]. These genes provide supporting functions to the virus for survival and replication by modulating the normal cellular pathways. The roles of these cellular molecules in various cellular pathways which contribute to viral survival in the infected host are discussed below.

7.1. v-Cyclin

KSHV-encoded v-cyclin is the viral homolog to cellular cyclin, most closely related to cyclin D [50]. Cellular cyclin D binds with cyclin dependent kinases (CDK6 and CDk4) [44], and this complex phosphorylates pRb releasing the transcription factor E2F. E2F, in turn, activates the transcription of S-phase genes, including cyclin E that is needed for G1–S phase transition. v-Cyclin like cellular cyclins are also able to phosphorylate pRb in vitro in complexes with CDKs. The v-cyclin CDK complexes are insensitive to CDK inhibitors (p16[INK4a], p21[CIP1] and p27[KIP1]). Thus, exogenous expression of v-cyclin from the infecting viral genome prevents CDK inhibitors-imposed G1 arrest, and stimulates entry into S-phase [29] (Fig. 4).

7.2. vBcl-2

vBcl-2, encoded by ORF16, is 60% identical to the cellular gene bcl-2, which has been shown to regulate programmed cell death by dimerization with other members of the family [53]. The vBcl-2 protein interacts with and inhibits the proapoptotic function of the cellular Bcl-2 family member, ensuring that the cell survives to allow for production of viral progeny.

7.3. vIRFs

KSHV encodes vIRF, which has significant sequence similarity to the human IRF family of proteins [52]. IFNs are either directly induced or are synthesized by T-lymphocytes or NK cells after virus infection. IFNs stimulate the anti-viral state in the target cell by modifying

signal transduction pathways, such as increased major histocompatibility complex I transcription, cell cycle shut down through induction of transcription of the CDK inhibitor p21 and also possibly via p53 independent apoptosis [54]. They can function as transcriptional factors by binding to the IFN-stimulated response element or the IFN-γ activation site in their promoters. KSHV-encoded IRFs share functional homology with human IRF-2, which is known to inhibit IFN-β signal transduction [55].

7.4. vGPCR

The KSHV-encoded vGPCR (ORF74) is one of the viral-encoded oncogenes and is most closely related to the IL-8 receptors CXCR1 and CXCR2 [29]. KSHV vGPCR has been shown to activate the phosphoinositide pathway (mitogenic signaling pathway) in COS-1 cells. Additionally, in vitro transfection of rat fibroblasts with vGPCR leads to cell proliferation [13]. Studies done before the discovery of KSHV showed that AIDS-associated KS expressed elevated levels of vascular endothelial growth factor which functions as an autocrine growth factor and is angiogenic [56] suggesting it could have a role in KS pathology. Additionally, vGPCR expressing NIH3T3 cells has been shown to induce tumor formation when injected into nude mice [57].

7.5. vMIP

KSHV-encoded chemokines, vMIP-I, vMIP-II and vMIP-III, show maximum similarity to cellular chemokines such as thymus activation-regulated chemokines, macrophage-derived chemokine and myeloid progenitor inhibitory factor-2 [58]. These viral-encoded macrophage inflammatory proteins have been shown to induce angiogenesis in chick chorioallantoic assays, suggesting a critical role in the pathogenesis of KS [59].

7.6. vIL-6

The KSHV-encoded IL-6 shares 24% identical amino acid sequence with human IL-6 [23]. Cultured KS cells have been shown to respond to recombinant hIL-6, suggesting vIL-6 has a role in KS pathogenesis. This is also supported by the fact that KS spindle cells express high affinity IL-6 receptor in vivo [23].

8. Treatment of KS

In classic KS patients with single lesions, simple excision is typically sufficient and can be used to treat recurrence [60]. However, patients with multiple lesions are often treated with radiation. In extensive or recurrent disease, patients can undergo a combination of surgery, radiation and chemotherapy. Complete remissions have also been shown to occur by chemotherapy including the use of vinblastine, bleomycin, doxorubicin alone or in combination therapy [1]. Endemic KS is usually treated with the identical drugs used for treating classic KS patients. In patients with immunosuppression-associated KS, the lesions typically regress with the reduction, modification or cessation of the immunosuppressive chemotherapeutic regimen. In AIDS-associated KS, highly active antiretroviral therapy (HAART) has been successfully used to treat patients [61]. IFN-α has also been used extensively for the treatment of AIDS-associated KS during the early periods of the disease. Additionally, IFN-α has proven to be more effective when used in combination with HAART [62].

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