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Structure and Function of Latency-Associated Nuclear Antigen

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Abstract

Latency-associated nuclear antigen (LANA) encoded by open reading frame 73 (ORF73) is the major latent protein expressed in all forms of KSHV-associated malignancies. LANA is a large (222–234 kDa) nuclear protein that interacts with various cellular as well as viral proteins. LANA has been classified as an oncogenic protein as it dysregulates various cellular pathways including tumor suppressor pathways associated with pRb and p53 and can transform primary rat embryo fibroblasts in cooperation with the cellular oncogene *Hras*. It associates with GSK-3 β , an important modulator of Wnt signaling pathway leading to the accumulation of cytoplasmic β -catenin, which upregulates Tcf/Lef regulated genes after entering into the nucleus. LANA also blocks the expression of RTA, the reactivation transcriptional activator, which is critical for the latency to lytic switch, and thus helps in maintaining viral latency. LANA tethers the viral episomal DNA to the host chromosomes by directly binding to its cognate binding sequence within the TR region of the genome through its C terminus and to the nucleosomes through the N terminus of the molecule. Tethering to the host chromosomes helps in efficient partitioning of the viral episomes in the dividing cells. Disruptions of LANA expression led to reduction in the episomal copies of the viral DNA, supporting its role in persistence of the viral DNA. The functions known so far suggest that LANA is a key player in KSHV-mediated pathogenesis.

1. Introduction

The search for an etiological agent associated with Kaposi sarcoma (KS) lesions led to the identification of Kaposi sarcoma-associated herpesvirus (KSHV) as a likely candidate as biological cofactor in KS development (Chang et al. 1994). KS lesions comprise layers of spindle cells expressing lymphatic endothelial, smooth muscle, macrophage, and/or dendritic cell marker (Weninger et al. 1999). During the onset of KS, only 10% of the spindle cells are KSHV positive, but they progress to approximately 90% during the late stage of KS lesions (Boshoff et al. 1995; Staskus et al. 1997; Dupin et al. 1999; Sturzl et al. 1999). These infected cells express the latency-associated nuclear antigen (LANA) (Rainbow et al. 1997; Sarid et al. 1999).

The KSHV genome comprises an approximately 140-Kbp-long unique coding region that is flanked by multiple terminal repeats of 801 bp with high GC content (Russo et al. 1996). It is believed that the long unique region encodes for approximately 90 open reading frames (ORFs), although it is not known whether each of these encodes for a functional gene product (Russo et al. 1996). On infection, KSHV enters a quiescent period known as latent infection whereby the viral genome circularizes and exists as nuclear episomes through

multiple host cell divisions (Renne et al. 1996). During this period, the virus limits gene expression to a specific subset of viral proteins, presumably to minimize the elicited host immune response (Zhong et al. 1996). This subset of protein includes LANA encoded by ORF73 (Kedes et al. 1997; Kellam et al. 1997; Rainbow et al. 1997), viral cyclin encoded by ORF72, and viral Fas-associated death domain (FAAD) interleukin-1 β -converting enzyme (FLICE) inhibitory protein (v-FLIP) encoded by ORF71. These adjacent genes are cotranscribed on two polycistronic mRNAs, LT1 (LANA, v-cyclin, v-FLIP) or LT 2 (v-cyclin, v-FLIP) (Dittmer et al. 1998; Sarid et al. 1999; Talbot et al. 1999).

Analysis of the infected cells for latent viral infection by immunofluorescence and immunohistochemistry indicated that the LANA protein is present in almost all KSHV-infected cells of KS, primary effusion lymphoma (PEL), and multicentric Castleman disease (MCD) (Rainbow et al. 1997; Dupin et al. 1999; Katano et al. 2000; Parravicini et al. 2000). However, v-cyclin and v-FLIP have been shown to be predominantly expressed in PEL cell lines (Platt et al. 2000; Low et al. 2001). As the majority of the tumor cells are latently infected (Boshoff et al. 1995; Staskus et al. 1997; Dittmer et al. 1998; Chang et al. 2000), latent, not lytic, antigens are believed to be involved in tumorigenesis, although additional studies have shown that some lytic antigens including K1, GPCR, and ORF37, the DNA exonuclease involved in RNA degradation and initiation of host cell gene expression (Lee et al. 1998; Couty et al. 2001; Jeong et al. 2001; Nador et al. 2001; Glaunsinger and Ganem 2004), are important mediators of KSHV-induced pathogenesis. Thus the viral components expressed during this phase of the viral life cycle are most likely the players for disease manifestation (Schulz 1999). This places attention on LANA as a major effector of KSHV pathogenesis.

2. Expression of LANA

2.1. Tissue-Specific Expression of LANA

KSHV becomes latent after infection in the majority of the cells it infects. During this quiescent stage of the viral life cycle, the virus exists in the nucleus as an episomal DNA (Renne et al. 1996). The viral DNA is typically copied by the host DNA replication machinery during cell division. During mitosis, KSHV DNA is tethered through specific sequences on the TR and to histone H1 on host chromatin via the KSHV-encoded LANA. Therefore, LANA is thought to play a central role in maintaining viral latency (Ballestas et al. 1999b; Cotter and Robertson 1999; Ballestas and Kaye 2001).

In more than 90% of infected tumor cells, KSHV is locked into latent infection, which is likely to be stringently controlled. Protein expression is restricted to only a few viral genes. Most prominent among these is LANA, which is involved in episome maintenance and transcriptional regulation associated with viral oncogenesis (Zhong et al. 1996; Kedes et al. 1997; Kellam et al. 1997; Rainbow et al. 1997). LANA mRNA transcription is shown to be under the control of LANA promoter (LANAp) (Sarid et al. 1998; Jeong et al. 2001; Renne et al. 2001). LANAp is active in the absence of other viral gene products in transient assays and in latent PEL and KS tumor cells (Jeong et al. 2001, 2004; Renne et al. 2001). ORF73 (encoding LANA), ORF72 (v-cyclinD), and ORF71 (vFLICE) are all expressed from the same locus in polycistronic, differentially spliced mRNAs whose transcription is coordinately regulated by a common promoter; these three ORFs form a major latent cassette (Cesarman et al. 1996; Dittmer et al. 1998; Sarid et al. 1999; Talbot et al. 1999; Grundhoff and Ganem 2001). Moreover, the LANAp is bidirectional, controlling the constitutive expression of the latent genes to the left but lytic, TPA-inducible expression of the K14 and ORF74/vGPCR to the right (Fig. 1). Interestingly, LANA can autoactivate its own promoter during latent infection, so ensuring that the levels of LANA are adequate for maintenance of latency (Jeong et al. 2001, 2004). Recently, Pearce et al. and Samols et al.

demonstrated the encoding of microRNAs from the latency transcriptional units, ORF71, -72, and -73 (Pearce et al. 2005; Samols et al. 2005). microRNAs are noncoding regulatory RNA molecules that bind to 3'-untranslated regions (UTRs) of mRNAs to either prevent their translation or induce their degradation, thereby regulating gene expression (Ambros 2004; Bartel 2004).

Generally, KSHV persists in KS and PEL tumor cells, which are of endothelial and B-cell origin, respectively. In asymptomatic carriers, KSHV is found in CD19-positive peripheral blood mononuclear cells (Mesri et al. 1996; Jeong et al. 2002). However, KSHV can infect various human cell lines such as primary fibroblasts, 293 cells, and human primary vascular endothelial cell preparations with low efficiency as well as animal cells such as baby hamster kidney (BHK) cells in tissue culture conditions (Renne et al. 1998; Lagunoff et al. 2002; Bechtel et al. 2003). The KSHV LANA protein is predominantly expressed in the context of primary infection, whereas lytic genes are infrequently transcribed (Sun et al. 1999; Paulose-Murphy et al. 2001). Similarly, LANAp is active in a wide range of cell lines after transient transfection in culture (Jeong et al. 2001, 2004). However, in a SCID-hu Thy/Liv mouse model of de novo KSHV infection, LANA mRNA was transcribed in CD19-positive B cells but not in the more abundant T-cell subsets (Dittmer et al. 1999). Dittmer's group also found that a region extending to -1299 bp upstream of the LANA transcription start site was able to drive *lac Z*-reporter gene expression in several lines of transgenic mice. In agreement with KSHV's natural tropism, reporter gene expression was detected in CD19-positive B cells but not in CD3-positive T cells. Surprisingly, reporter gene expression was also detected in the kidney and in the liver at a lower level. In contrast to KS tumors, transgene expression was localized to kidney tubular epithelium rather than vascular endothelial cells (Jeong et al. 2002). This suggests that this particular promoter fragment contains all *ci s*-regulatory elements sufficient for B-cell specificity but is likely to lack those required for endothelial specificity. These in vivo studies reflect a conundrum in KSHV biology: In culture, KSHV is able to infect a variety of cell types indiscriminantly, whereas in healthy latent carriers KSHV is found in B lymphocytes (Lagunoff et al. 2002). The transgenic mouse experiments suggest that there are tissue-restricted mechanisms regulating LANA gene expression, providing one possible explanation for B cell-specific viral persistence (Jeong et al. 2002).

2.2. Levels of LANA mRNA in Infected Cells

Like other herpesvirus, KSHV also displays a tightly controlled program of gene expression. KSHV can enter one of two modes of infection: (a) latent infection, in which the viral genome persists in its host cell with restricted gene expression without cell destruction (Zhong et al. 1996), and (b) lytic infection, which produces virion progeny and destroys the host cell (Sarid et al. 1998). Lytic infection, in turn, can be divided into immediate-early, early, and late stages. With KSHV DNA fragments as a probe, three classes of differentially transcribed messages were defined in a KSHV-infected PEL cell line (Sarid et al. 1998). Class I mRNAs can be detected in untreated cultures and are not increased after 12-*O*-tetradecanoylphorbol-13-acetate (TPA) treatment, which reactivates the KSHV lytic replication cycle. Class II mRNAs can be detected in untreated cultures but are greatly increased by TPA treatment. Class III mRNAs can only be detected in TPA-treated cultures (Sarid et al. 1998). Translated into the customary herpesvirus classification, class III mRNAs correspond to lytic transcripts and class I mRNAs correspond to latent transcripts. Class II mRNAs appear to be a unique set of transcripts, because it is not clear whether the mRNAs detected in untreated cultures stem from the few percent of cells that undergo spontaneous lytic reactivation in culture. KSHV-specific cDNA arrays have also confirmed and expanded these data (Jenner et al. 2001; Paulose-Murphy et al. 2001). Although KSHV lytic transcription appears to be an all-or-nothing response, which proceeds to completion

once orf50 (RTA) is expressed, KSHV can enter very distinct latency programs in terms of temporal regulation (latency type one or two) and tissue specificity (KS endothelial vs. B cell lineage). The type one latency mRNAs are resistant to the effects of the known chemical inducers such as ionomycin or butyrate, as well as TPA (Fakhari and Dittmer 2002). This suggests that LANAp, the promoter for LANA, v-FLIP, and v-cyclin, and the LANA-2/v-IRF-3 promoter share common regulatory features. The fact that latency type one mRNAs do not increase on viral reactivation in BCBL-1 cells suggests that their expression might be latency specific and that latency type one mRNAs might be expressed in latently infected cells to the exclusion of lytic mRNAs. Alternatively, latency type one mRNAs might be constitutively transcribed, but their promoters are unaffected or protected from the orf50-mediated upregulation of neighboring lytic transcription units (Fakhari and Dittmer 2002).

A number of studies showed that the mRNA level for LANA is unchanged after viral reactivation induced by TPA (Dittmer et al. 1998; Dittmer 2003). However, Jung and colleagues demonstrated opposite results by using a RTA tetracycline-inducible BCBL1 cell line (Nakamura et al. 2003). Under induced conditions, RTA expression is turned on, and in this system they were able to monitor changes in all viral transcripts. Interestingly, LANA mRNA levels are dramatically upregulated (Nakamura et al. 2003). RTA was shown to play a critical role in KSHV lytic reactivation, and forced expression of RTA was shown to initiate the full program of lytic replication (Lukac et al. 1998, 1999). These results suggest that LANA expression level may be changed during the natural reactivation process. In support of these studies, Chandran and colleagues showed that LANA transcripts were turned on by 48 h after infection (Krishnan et al. 2004).

3. Structure and Characterization of LANA

Sera obtained from HHV-8-infected patients were shown to recognize a specific nuclear antigen in latently infected PEL cell lines or KS tissue. This was characterized by a punctate nuclear immunofluorescence pattern (Lennette et al. 1996) (Fig. 2). Screening of cDNA libraries with serum from a HHV8-positive patient identified this nuclear antigen as the product of the viral gene open reading frame 73 (ORF73), and the encoded protein was designated LANA (Kedes et al. 1997; Kellam et al. 1997; Rainbow et al. 1997). LANA is expressed from a latently controlled 5.32-kb transcript that also encodes the viral cyclin (v-Cyc) and v-FLIP (Dittmer et al. 1998). The 5.32-kb latent transcript is spliced to form a 1.7-kb transcript that encodes only v-Cyc and v-FLIP (Kellam et al. 1997; Dittmer et al. 1998). The predicted LANA protein is 1,162 amino acids (aa) and has a theoretical molecular mass of 135 kDa. In contrast to LANA's predicted molecular size, this protein has a much higher molecular size of 220–230 kDa when analyzed by Western blotting (Gao et al. 1996; Kellam et al. 1997; Rainbow et al. 1997). LANA is divided into three distinct domains: an N-terminal 337-amino acid domain; an extremely hydrophilic central domain of 585 aa consisting of multiple repeat elements predominantly containing the charged polar amino acids glutamine, glutamic acid, and asparagine; and a C-terminal 240-amino acid domain (Russo et al. 1996). In contrast to the central domain, the N- and C-terminal domains are rich in basic amino acids (Fig. 3). The differently charged domains of the protein may in part explain the aberrant running of LANA on sodium dodecyl sulfate (SDS)-polyacrylamide gels.

To date, a number of studies have investigated the structural and functional aspects of LANA. In strain BC-1, LANA contains 1,162 aa (Russo et al. 1996) and has an apparent molecular mass of 224–234 kDa on SDS polyacrylamide gels (Rainbow et al. 1997). However, its size varies greatly among HHV-8 isolates, mainly because of variations in length that affect the large internal acidic domain (Neipel et al. 1997; Rainbow et al. 1997; Gao et al. 1999; Glenn et al. 1999; Zhang et al. 2000). Sequence alignments of LANA from

BC1, BBG1, KS1, and KS2 cell lines showed that LANA can be divided into two conserved regions, spanning aa 1 to 337 (the unique left region) and 798 to 1036 (the unique right region), that are separated by an internal region composed mainly of a limited number of repeated motifs (Piolot et al. 2001). Nucleotide alignments revealed that only four nucleotide positions are variable in the unique left region, whereas the unique right region was completely conserved (Piolot et al. 2001). In contrast, high variability was observed in the central acidic domain, at both the nucleotide and amino acid levels. The region encoding aa 338–797 is composed of a series of short conserved tandem repeats whose number is characteristic of each HHV-8 isolate. This region is interrupted by a conserved nonrepeated sequence (the unique central region) encompassing aa 439–452 (Piolot et al. 2001).

Studies have shown that LANA is localized in the nuclei of KSHV latently infected cells and that it interacts with the border of heterochromatin during interphase and with chromosomes during mitosis (Szekely et al. 1998, 1999; Ballestas et al. 1999b; Cotter and Robertson 1999). A unique nuclear localization signal (NLS) sequence was mapped within aa 24–30 of LANA (Szekely et al. 1999; Schwam et al. 2000; Piolot et al. 2001). The LANA NLS is highly homologous to the NLSs that have been identified in EBV EBNA1 (Schwam et al. 2000). In particular, these sequences have a phosphorylation site for protein kinase A and a cdc2-type kinase in common. Because phosphorylation of residues close to or within the NLS has been shown to modify the nuclear import of numerous cellular proteins, this suggests that LANA nuclear transport may be regulated by a similar kinase(s), notably during the course of the cell cycle (Boulikas 1993). Except for the N terminus, which was shown to be localized to nucleus, Wilson and colleagues demonstrated that both the N and C termini of LANA can localize to the nucleus. They showed that the N terminus gives a diffuse pattern in interphase nuclei typical of many transcription factors; however the C terminus accumulates as discrete nuclear speckles reminiscent of the punctate pattern shown by full-length LANA (Schalling et al. 1995; Friborg et al. 1999; Glenn et al. 1999).

It has also been shown that LANA self-associates to form dimers in the absence of viral DNA or other viral gene products (Schwam et al. 2000). Self-association is mediated by the C terminus of LANA, and, interestingly, the sequences required for self-association of LANA are conserved. The amino acid sequence of the C terminus of KSHV LANA with the similar regions of LANA homologs encoded by four other rhadinoviruses, ateline herpesvirus-3 (Ensler et al. 1997), herpesvirus saimiri (Albrecht et al. 1992), macaque rhadinovirus 17577 (Searles et al. 1999), and murine herpesvirus 68 (Virgin et al. 1997), when compared showed that the greatest conservation of the C-terminal sequence corresponds to residues 934–1072 of KSHV LANA, with a more limited conservation between residues 885 and 922 (Russo et al. 1996). Clearly, the C terminus of LANA (residues 884–1089) is required for self-association and includes the majority of the residues shown to be conserved between members of the Rhadinovirus family (Schwam et al. 2000).

Like EBNA1 of EBV, LANA tethers the viral episomes to host chromosomes, ensuring that the viral genome is segregated to daughter cells on mitosis (Cotter and Robertson 1999; Schwam et al. 2000). LANA interacts with the mitotic chromosomes in KSHV-infected cells. The major chromosome-binding site of LANA was mapped to the N terminus between aa 1 and 32 (Schwam et al. 2000). This region also contains the LANA NLS; therefore it is possible that nuclear targeting and binding to mitotic chromosomes were functionally related (Schwam et al. 2000).

4. The Multifunctional Role of LANA

4.1. LANA as a Transcriptional Modulator

Numerous studies from a number of laboratories have demonstrated that LANA can activate as well as repress transcription (Groves et al. 2001; Hyun et al. 2001; Jeong et al. 2001; Knight et al. 2001; Lim et al. 2001; Renne et al. 2001; An et al. 2002). LANA has also been shown to autoactivate transcription of the major latent promoter (Renne et al. 2001; Jeong et al. 2004), suggesting a role in maintenance of expression of these latent transcripts while the majority of the viral genome remains silent. Transcriptional activation by LANA is probably independent of direct DNA binding as no specific LANA binding sequences have been identified in the major promoter elements (Russo et al. 1996). LANA activation of transcription is directed by a wide array of simple, synthetic promoters containing binding sites for a range of cellular proteins including ATF, AP-1, CAAT, or Sp1, linked to a TATA box (Renne et al. 2001). Although the TATA box alone was shown to be activated by LANA in these studies (Renne et al. 2001), activation of the cellular IL-6 promoter by LANA required both a TATA box and an upstream AP-1 element (An et al. 2002). Additionally, activation of the HIV-1 LTR in BJAB cells requires both the TATA box and the core enhancer elements (NF-IL-6, Ets, NF- κ B, and Sp1 sites) (Hyun et al. 2001). However, LANA can activate the HIV-1 LTR independently of the core enhancer if the Tat protein is coexpressed; this effect is mediated by the C-terminal 400 aa of LANA, which includes the third repeat region (Hyun et al. 2001). Truncated LANA containing the internal repeat domain (IRD) plus the C terminus activates the transcription of an EBV latency promoter containing PU.1, ATF, and Sp1 elements (Groves et al. 2001). Mechanistically, LANA can modify the DNA binding activity of Sp1 to activate the transcription of the telomerase reverse transcriptase promoter (Knight et al. 2001). Furthermore, LANA was shown to interact with Sp1 directly as well as associated in KSHV-infected cells, suggesting an upregulation of hTERT by directly interacting with Sp1 at the transcription domains (Verma et al. 2004).

LANA can also contribute to broad repressive effects on transcription. In Cos cells, LANA represses the transcriptional activity of NF- κ B. Additionally, repression of the HIV-1 LTR suggested that the interaction of LANA with the cellular transcription apparatus is cell specific (Renne et al. 2001). LANA also represses transcription controlled by the ATF4/CREB2 protein, independently of ATF4 DNA binding. This effect is mediated by LANA aa 751–1162, which includes the third repeat region (Lim et al. 2001). Similarly, the first acidic repeat (aa 340–431) of LANA is one of two domains required to competitively bind the cellular cyclic AMP-responsive element binding protein (CREB) binding protein (CBP) and block its histone acetyltransferase activity, thus inhibiting the ability of CBP to coactivate transcription with c-fos (Lim et al. 2001). This mechanism reflects the general ability of LANA to repress transcription driven by other cellular and viral CBP-dependent transactivators, including the KSHV lytic switch protein ORF50/Rta (Gwack et al. 2001). To determine the role of LANA on cellular pathways, an overexpression system followed by a microarray analysis revealed the induction of a large number of genes in LANA-expressing cells including pRb and p53 signaling molecules (An et al. 2005).

4.1.1. LANA Contributes to Maintenance of Viral Latency by Regulating RTA Function

4.1.1.1. LANA Represses RTA Expression: Recently, studies in our laboratory showed that LANA is capable of maintaining viral latency through repression of the transcriptional activity of RTA (replication and transcription activator) promoter (Lan et al. 2004). RTA is encoded by ORF50 of KSHV and is an immediate-early protein. Exogenous expression of RTA from a heterologous promoter or induction of endogenous RTA expression with

chemical inducers can initiate lytic reactivation of KSHV (Lukac et al. 1998, 1999). HHV-8 RTA is the key transcriptional regulator that controls the switch from viral latency to lytic replication and is sufficient to drive the viral lytic cascade to completion, resulting in the production of viral progeny (Lukac et al. 1998, 1999). The RTA protein is made as an IE gene product; it autoregulates its own expression, and activates the transcription of various viral and cellular genes (Deng et al. 2000). It was shown that LANA downmodulates Rta's promoter activity in transient reporter assays, thus repressing the Rta-mediated transactivation (Lan et al. 2004). This eventually resulted in a decrease in the production of KSHV progeny (Lan et al. 2004). LANA physically interacts with Rta in vitro and associates with RTA in KSHV-infected cells (Lan et al. 2004). These results demonstrated that LANA is capable of inhibiting viral lytic replication by repressing transcription and antagonizing the function of RTA (Lan et al. 2004). Therefore LANA provides a critical role in controlling the switch between viral latency and lytic replication (Lan et al. 2004). Similarly, Schafer and colleagues in a recent report also indicated that the LANA homologue in HVS can repress the expression of ORF50 encoded by HVS (Schafer et al. 2003). Most recently, DeWire and Da-mania also showed that the LANA homologue of rhesus monkey rhadinovirus (RRV) can dramatically decrease the transactivation function of RRV Orf50 (RTA) and inhibit RRV lytic replication in vitro (DeWire and Damania 2005). Interestingly, a recent report showed that KSHV could undergo spontaneous lytic replication, and RTA transcription level was increased when LANA expression was knocked down with specific siRNA (Godfrey et al. 2005). These findings provide one explanation as to why KSHV is predominantly maintained in the latent state in infected cells as well as why after the primary lytic infection, KSHV rapidly establishes latent infection. Moreover, it may be a common and conserved mechanism whereby LANA inhibits lytic reactivation through downmodulation of the immediate-early transactivator, RTA expression. Further, it has been shown that LANA downregulates RTA's promoter activity through the RBP-J κ binding sites within the promoter (Lan et al. 2005a). RBP-J κ is a sequence-specific DNA-binding protein in the CSL/CBF-1 family, normally thought to function as a major repressor of transcription in response to activation of the Notch pathway signaling. Our results once again showed that viral proteins can regulate gene expression through direct targeting of important cellular signaling pathways. Interestingly, Liang and colleagues also demonstrated that RTA can functionally interact with RBP-J κ and mimic cellular Notch function to activate lytic gene expression (Liang et al. 2002; Liang and Ganem 2003).

4.1.1.2. RTA Upregulates LANA After De Novo Infection: Most recently, it has been shown that RTA is detected in association with KSHV viral particles (Bechtel et al. 2005; Lan et al. 2005a, 2005b). This raises the possibility that RTA may contribute to latency establishment by inducing LANA expression upon entry. Interestingly, it was shown that RTA activates the LANA promoter and induces LANA expression in transient transfection experiments (Lan et al. 2005b). Moreover, the transcription of RTA correlates with that of LANA during the early stages of de novo infection of KSHV and LANA transcription is responsive to induction of RTA expression (Lan et al. 2005b). Additionally, the RBP-J κ binding site within the LANA promoter is also shown to be critical for regulation by RTA known to be associated with a number of cellular regulatory proteins including RBP-J κ . In RBP-J κ -knockout cells KSHV was unable to establish latent infection efficiently compared to wild-type cells (Lan et al. 2005b). These findings therefore suggest that RTA can contribute to the establishment of latency through activation of LANA expression in the early stages of infection. This represents a feedback loop whereby LANA and Rta regulate each other and is likely to be, at least in part, a key event in establishment of KSHV latent infection. A potential model can be proposed that suggests a feedback regulatory loop between RTA and LANA and implies that the interplay between RTA and LANA could be the master control for the switch between lytic replication and latency of KSHV utilizing the

major cellular Notch signaling pathway through targeting of the RBP-J κ effector protein (Fig. 4).

4.2. Deregulation of Cellular Factors by LANA

4.2.1. Role of LANA on Action of GSK-3 β and β -Catenin in Cell Cycle

Deregulation—LANA, which is expressed in all forms of KS tumors, modulates distribution of glycogen synthase kinase (GSK)-3 β , a negative regulator of β -catenin and Wnt signaling (Fujimuro et al. 2003). In normal cells cytosolic amounts of β -catenin are normally limiting because it leads to targeted degradation after phosphorylation with GSK-3 β (Polakis 2000). The nuclear effector protein β -catenin binds to the cytoplasmic domain of E-cadherin, which is the transmembrane protein present at adhesion junctions, and also to the cytoplasmic complex comprised of the serine-threonine kinase GSK-3 β , the tumor suppressor adenomatous polyposis coli (APC) and the scaffold protein Axin (Polakis 2000). Association of β -catenin with the above complex results in phosphorylation of β -catenin by GSK-3 β through association with the F-box protein β -TrCP leading to ubiquitination and thus proteosomal degradation by the specific E5 ligases (Kitagawa et al. 1999). Activation of Wnt signaling by binding of Wnt ligand to a frizzled (Fz)/low-density lipoprotein receptor-related protein (LRP) complex activates the cytoplasmic protein Disheveled (Dsh in *Drosophila* and Dvl in vertebrates), possibly through phosphorylation by casein kinase 1 (CK1) and casein kinase 2 (CK2) (Willert et al. 1997; Sakanaka et al. 1999). Dsh/Dvl then inhibits the activity of the multiprotein complex β -catenin-Axin-APC-GSK-3 β , which targets β -catenin for proteosomal degradation by phosphorylation, resulting in accumulation of cytosolic forms of β -catenin (Polakis 2000). Stabilized β -catenin translocates into the nucleus and binds to members of the T-cell factor (Tcf)/lymphoid enhancing factor (Lef) family of DNA binding proteins, leading to transcription of Wnt target genes (Boshoff 2003).

Fujimuro and Hayward reported increased levels of β -catenin in PEL cells (BC2, BC3, and BCBL1) (Fujimuro et al. 2003; Fujimuro and Hayward 2004). Immunohistochemical detection of β -catenin showed elevated expression of β -catenin in KS tumors compared to the surrounding normal tissues (Fujimuro et al. 2003). LANA, which is primarily expressed in all KSHV-infected cells, induced the accumulation of β -catenin (Fujimuro et al. 2003). The accumulation of β -catenin is possibly through interaction of LANA with GSK-3 β , which is important for phosphorylation of β -catenin and its degradation. LANA changes the distribution of GSK-3 β from predominantly cytoplasmic to nuclear, thus preventing phosphorylation of cytoplasmic β -catenin, which in turn translocates to the nucleus and forms a complex with the Tcf and Lef family of transcription factors to activate genes containing Tcf and Lef binding sites (He et al. 1998; Barker et al. 2000; Fujimuro and Hayward 2003, 2004; Fujimuro et al. 2003). These target genes include *MYC*, *JUN*, and *CCND1*, known to be critical in regulating cell proliferation (cyclin D1) (He et al. 1998; Boshoff 2003; Fujimuro et al. 2003) (Fig. 5).

4.2.2. LANA Regulates p53 Activity—p53 was the first identified tumor suppressor gene, and the induction of this pathway eliminates or inhibits the proliferation of abnormal cells, thereby preventing neoplastic development (Hollstein et al. 1991; Friend 1994; Levine 1997). Mutations identified in the p53 gene are estimated to occur in 50% of all cancers, and loss of p53 function leads to cell transformation and oncogenesis (Hollstein et al. 1991; Levine 1997). Besides mutation in the gene, several viral oncoproteins have been shown to interact with p53 and modulate its transcriptional activity (Chen et al. 1996; Ko and Prives 1996). HPV-16-encoded E6 binds with ubiquitin ligase to mediate p53 ubiquitination and degradation (Werness et al. 1990). SV40 large T antigen modulates p53-mediated apoptosis by physically interacting with and inactivating p53 (Jay et al. 1981). More recently, Nabel

and colleagues have shown that LANA interacts with p53 and downregulates its transcriptional activity as well as blocking its ability to induce apoptosis (Friborg et al. 1999). Modulation of p53 transcriptional activity by LANA is neither through degradation nor inhibition of its ability to bind DNA. This raises the possibility that additional factors are required for its function (Friborg et al. 1999). The region of LANA that binds to p53 was mapped to aa 441–1162, which is further narrowed down to aa 751–1162 (Borah et al. 2004) (Fig. 1). The herpesvirus positional homologue of LANA also binds to p53 and most probably performs a similar function (Borah et al. 2004). The domain of p53 that maps to bind KSHV-encoded LANA and its HVS ORF73 homologue is the tetramerization domain essential for enzymatic activity, but the presence of LANA did not affect the tetramerization of p53, suggesting that inactivation of p53 function by LANA may involve another potential mechanism besides that which involves tetramer disruption (Borah et al. 2004). Recently, Si and Robertson reported that LANA-expressing cells had increased chromosomal instability, detected by the presence of increased multinucleation, micronuclei, and aberrant centrosomes (Si and Robertson 2006). They proposed that repression of p53 functional activity contributes to the chromosomal instability, thereby facilitating KSHV-mediated pathogenesis (Si and Robertson 2006).

4.2.3. LANA Inhibits Rb-Induced Growth Arrest—Retinoblastoma (pRB) acts as a negative regulator of cell cycle progression by blocking the cells from entering into S phase from G₁ (Sherr and Mc-Cormick 2002). In early G₁, pRB is hypophosphorylated and associates with specific members of the E2F transcription factor, finally resulting in transcriptional repression (Kiess et al. 1995). Cell cycle progression from G₁ to S phase occurs when pRB is hyperphosphorylated by complexes of D-type cyclins/CDK4/CDK6, thereby releasing the E2F transcription factor. This results in activation of the DNA replication machinery and nucleotide synthesis to prepare the cell for division (Dyson 1998). LANA has been shown to bind and inactivate pRb, thereby upregulating transcription of the promoter containing the cell cycle transcription factor E2F DNA-binding sequences (Radkov et al. 2000). Exogenous expression of LANA in SAOS-2 cells overcomes pRb-induced growth arrest and drives the cells into S phase (Radkov et al. 2000). LANA can transform primary rat embryo fibroblasts and make them tumorigenic in cooperation with the cellular oncogene Harvey rat sarcoma viral oncogene homologue (*Hras*) (Radkov et al. 2000). Additionally, the LANA homologue of herpesvirus saimiri ORF73 can also bind to pRB, resulting in upregulation of E2F responsive promoter activity (Borah et al. 2004) (Fig. 6).

4.2.4. LANA Associates with RING3/Brd2—RING3 is a ubiquitously expressed group of proteins in mammalian cells (Beck et al. 1992; Apone et al. 1996). They are bromodomain-containing proteins that include the SWI/SNF-type chromatin remodeling proteins that have elevated activity in human leukemia (Denis and Green 1996). LANA binds to RING3 through the extraterminal (ET) domain, characteristic of fsh-related proteins, suggesting a highly conserved function in terms of protein-protein interactions (Beck et al. 1992; Nomura et al. 1994; Jones et al. 1997). Proteins belonging to this group have been shown to be involved in cell cycle control. Interaction of RING3/Brd2 shown with nuclear extract protein complex has been hypothesized to promote G₁-S transition (Denis et al. 2000; Guo et al. 2000). The interaction between LANA and RING3 leads to the phosphorylation of serine and threonine residues in the C terminus of LANA, between residues 951 and 1107 (Platt et al. 1999). This group of proteins also includes the yeast BDF proteins that regulate transcription through interaction with general transcription factors (Guo et al. 2000). RING3 localizes to euchromatin regions in the interphase nucleus even in the absence of KSHV viral genome and is released to the cytoplasm during mitosis. In KSHV-infected cells most of the RING3 proteins colocalized with LANA, suggesting that

RING3 may contribute to KSHV genome persistence by local euchromatin microenvironment around the viral episomes tethered to the heterochromatin modulating transcription or promoter replication-segregation of the KSHV genome (Mattsson et al. 2002). Very recently RING3/Brd2 binding domain of LANA was mapped lying between aa 1007 and 1055 (Viejo-Borbolla et al. 2005). Mutants of LANA capable of supporting replication and dimerization were able to interact with RING3/Brd2, suggesting that RING3 may be important for interaction of C-terminal domain of LANA with heterochromatin and its functional activities (Viejo-Borbolla et al. 2005).

4.2.5. The Interferon-Inducible Protein MND1 Binds to LANA—MND1 is a nuclear protein expressed selectively in hematopoietic cells and has been shown to be induced by interferon (IFN) (Johnstone and Trapani 1999). This protein, like other proteins of this family, human IFI16, AIM2 and murine p202, p203, 204, and D3 protein (hematopoietic interferon-inducible nuclear protein), contains a conserved 200-aa region (Johnstone and Trapani 1999). LANA binds to MND1 through its amino-terminal region (aa 22–274) (Fukushi et al. 2003). This region of LANA has also been shown to be involved in transcriptional repression (Lim et al. 2000; Schwam et al. 2000). LANA colocalizes with MND1 in PEL cells and also is overexpressed in HEK cells (Fukushi et al. 2003). The primary function of IFN in infected cells is the inhibition of the growth of virus-infected cells, but exogenous expression of IFN did not affect growth of KSHV-infected cells (Fukushi et al. 2003). These studies suggest that MND1 alone is not enough for IFN-induced growth inhibition of KSHV-infected cells (Fukushi et al. 2003).

4.2.6. LANA Associates with the Acetyl Transferase pCBP and p300—CREB binding protein (CBP) and p300 were both identified through protein-protein interaction assays. CBP was identified in association with the transcription factor CREB (Chrivia et al. 1993) and p300 through its interaction with the adenoviral transforming protein E1A (Qiu et al. 1998). These proteins share high sequence homology and have been shown to be involved in a variety of cellular functions including modulation of tumor suppressor pathways (Wu et al. 2002; Schiller et al. 2003; Iyer et al. 2004). CBP and p300 may also serve as tumor suppressors through more indirect mechanisms; for example, CBP has been shown to be a negative regulator of the Wnt pathway in *Drosophila* (Waltzer and Bienz 1998), and activation of the Wnt pathway induces *c-myc* and cyclin D1 (at least in mammalian cells) (He et al. 1998). Therefore the loss of CBP would be expected to activate Wnt targets, with a resultant induction of tumorigenesis (Fig. 7).

LANA has been shown to bind with CBP and interferes with the interaction of CBP with *c-Fos*, a representative C/H3 region binding cellular transcription factor, in vivo and in vitro (Lim et al. 2001). Additionally, LANA inhibits transcriptional activity and also the in vitro histone acetyltransferase activity of CBP, suggesting that LANA modulates the global transcriptional activities of infected cells at least in part through interaction with CBP (Lim et al. 2001).

4.2.7. LANA Induces Expression of the Id Proteins Involved in Regulating Cell Differentiation—Id proteins belong to a family of four helix-loop-helix (HLH) proteins (Id-1 to Id-4) that were initially recognized as growth factor-inducible genes that inhibited cell differentiation (Benezra et al. 1990). These proteins are also referred to as inhibitors of DNA binding (Id) because they lack the basic amino acid sequence characteristic of basic bHLH transcription factors necessary for DNA binding. They also form heterodimers with transcription factors, inhibiting their ability to bind DNA (Benezra et al. 1990; Norton 2000). Recently various biological roles have been assigned to the Id family of proteins, including cell cycle regulation, embryonic development, cell death, and tumorigenesis (Nickoloff et al. 2000). These recently described functions may be through interaction with

non-HLH proteins including the pocket domain proteins, pRb, p107 and p130, MDA1, and ETS transcription factors (Hollnagel et al. 1999; Yates et al. 1999). Id proteins also promote cell progression through dysregulation of expression of p21, inhibitor of the cell cycle-dependent kinase (CDKI). Moreover, Id-1 regulates cell proliferation, possibly through direct interactions with the p16 promoter and/or inactivation of ETS2 transcription factors (Alani et al. 2001). Expression profile of Id-1 in human tumors, including squamous cell carcinoma and endometrial, breast, and cervical cancers, showed a significant level of expression compared to normal cells (Langlands et al. 2000; Schindl et al. 2001; Tang et al. 2003). Higher levels of expression have also been found in invasive or high-grade tumors, indicating a possible correlation with the more aggressive form of the disease (Lin et al. 2000). Overexpression of Id-1 in keratinocytes and endothelial cells (ECs) has been shown to delay onset of cellular senescence (Tang et al. 2003). KS tumors showed elevated levels of Id-1 expression, and infection with KSHV drastically increased Id-1 expression (Tang et al. 2003). Ectopic expression of LANA in endothelial cells increased Id-1 expression, suggesting that LANA may drive cell proliferation by inducing Id-1 expression (Tang et al. 2003).

4.3. Role of LANA in Episome Maintenance

Persistence of KSHV through successive rounds of cell division in new daughter cells is enhanced by the partitioning of the viral DNA to host daughter cells during mitosis to prevent loss of the viral DNA after the cell divides. Studies have demonstrated that LANA associates with the KSHV genome in infected cells and colocalizes with the viral genome both in interphase nuclei and on mitotic chromatin in a punctate pattern (Ballestas et al. 1999b; Cotter and Robertson 1999). The ability of LANA to support persistence of the KSHV genome is crucial for the establishment of latency. LANA has been shown to target heterochromatin-associated nuclear bodies and selectively associates with human chromatin in human mouse hybrids containing a single fused nucleus (Szekely et al. 1999). The presence of LANA was shown to be essential for the long-term maintenance of the KSHV-derived Z6 cosmids (Ballestas et al. 1999b; Cotter and Robertson 1999). The above reports showed colocalization of LANA with the viral genome in the nucleus, demonstrated its role in episomal persistence, and additionally suggested its role in partitioning of the viral genome during mitosis. Recombinant KSHV virus cloned in a bacterial artificial chromosome (BAC36ΔLANA) disrupted for LANA expression did not maintain viral episome, and the cells became virus free after 2 weeks of selection, confirming LANA's role in viral episome maintenance (Ye et al. 2004). Recently, Godfrey and colleagues used a short hairpin RNA (shRNA) approach to knock down the expression of the oncogenic latent gene cluster including LANA (Godfrey et al. 2005). Independent silencing of LANA did not completely knock down expression of LANA or v-cyclin but reduced it to 20% compared to control treatments. The reduction in the expression of LANA gradually reduced KSHV copy number until it achieved a lower but steady copy number (Godfrey et al. 2005). Indirect 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 stronger binding compared to the entire genome (Cotter and Robertson 1999). A small 2-Kbp region at the left end was shown to have strongest binding to LANA, and the binding sequence was narrowed down to a 13-bp cognate sequence in the TR region of the KSHV genome (Ballestas and Kaye 2001; Cotter et al. 2001; Renne et al. 2001). The binding region of LANA was initially mapped to the last 200 aa of the carboxy terminus and is now further mapped to residues 996–1139 (Komatsu et al. 2004). Scanning deletions within this region ablated both LANA oligomerization and DNA binding, consistent with a requirement for oligomerization to bind DNA (Komatsu et al. 2004; Srinivasan et al. 2004). A region of the KSHV genome at the left end as well as a single and three copies of the TR elements persisted in cells stably expressing LANA in *trans*, suggesting that this region in

fact contained the *cis*-acting DNA element (Cotter and Robertson 1999; Ballestas and Kaye 2001; Renne et al. 2001). However, this does not exclude other regions of the genome as alternate elements capable of functioning as origins of replication during persistent infection. Interestingly, LANA showed affinity to two other regions of the KSHV genome, albeit to a lesser degree than the left end (Cotter and Robertson 1999). LANA was also shown to bind to histone H1 but not core histones and to tether the viral episomes to the host chromatin (Cotter and Robertson 1999). A deletion in the chromosome binding domain (CBD), aa 5–22 of the N-terminal region of LANA, abolished episomal maintenance but was restored by replacing the mutation with histone H1 protein (Shinohara et al. 2002). LANA therefore simultaneously associates with mitotic chromosomes via the CBD of its N terminus and through binding of its C terminus to the KSHV TR (Ballestas et al. 1999a; Piolot et al. 2001). These domains mediate chromosomal tethering, which may involve interactions with cellular proteins including the methyl CpG binding protein (MeCP2), heterochromatin proteins (HP-1) at N terminus, and DEK at C terminus of LANA protein (Krithivas et al. 2002) (Fig. 8). These studies demonstrate that LANA can function similar to EBNA1 in maintenance of the EBV episome in the infected cell (Cotter and Robertson 1999).

4.4. Viral DNA Replication

Replication of viral DNA is required for efficient segregation of viral episomes. LANA has been shown to be essential for the replication of the viral cosmid clone along with tethering to the host chromosomes (Ballestas et al. 1999b; Cotter and Robertson 1999). The TR elements of the KSHV genome have been proposed to be the potential site of initiation of replication by various groups using short-term replication assays (Hu et al. 2002; Lim et al. 2002; Grundhoff and Ganem 2003; Barbera et al. 2004; Komatsu et al. 2004; Hu and Renne 2005), although the replicative potential of other regions has not been thoroughly evaluated. Plasmids containing TR elements have been shown to replicate in LANA-expressing cells (Hu et al. 2002; Lim et al. 2002; Grundhoff and Ganem 2003; Hu and Renne 2005). Renne and colleagues performed deletion mapping of the TR region to identify minimal replicator sequences, and it was shown that a 71-bp-long minimal replicator region contained two distinctive sequence elements, LANA binding sites (LBS1/2). Additionally, adjacent 29- to 32-bp-long GC-rich sequences were essential for replication of the TR elements and thus could be termed a replication element (Hu and Renne 2005). In terms of proteins required for the replication of the viral episome, a number of cellular proteins have been proposed to be involved in this process. Human origin recognition complexes (hsORCs) have been shown to interact with LANA in *in vitro* experiments, most probably similar to EBV encoded EBV, which is believed to recruit cellular replication machinery at the Ori site (Dhar et al. 2001; Schneider 2001; Ritzi et al. 2003). The detailed mechanism of replication in KSHV is not well understood. However, recently it was shown that KSHV forms chromatin structures and the latent replication origin within the TR is bound by the LANA-associated proteins CBP, double-bromodomain-containing protein 2 (BRD2), and origin recognition complex 2 protein (ORC2) (Stedman et al. 2004). This region is enriched in hyperacetylated histones H3 and H4 relative to other regions of the latent genome (Stedman et al. 2004). In slight contrast to previous studies, chromatin immunoprecipitation assays performed in our lab have shown that the association of cellular replication machinery proteins ORC2 and MCM3 occurs at various regions along the KSHV genome, suggesting that additional regions of the KSHV genome may be capable of functioning as origins of replication (Verma 2006).

5. Overview of LANA's Role in KSHV Pathogenesis

Numerous studies over the last 10 years have shown that LANA is a multifunctional protein ubiquitously expressed in KSHV-infected cells (Ballestas et al. 1999b; Cotter and Robertson

1999; Friborg et al. 1999; Radkov et al. 2000; An et al. 2002; Boshoff 2003; Cannon et al. 2003; Fujimuro et al. 2003; Fukushi et al. 2003; Verma and Robertson 2003; Borah et al. 2004; Cloutier et al. 2004; Lan et al. 2004; An et al. 2005). LANA modulates various cellular pathways both positively and negatively to drive cell proliferation. LANA also downregulates tumor suppressor pathways regulated by p53 and pRB as discussed earlier in this chapter. Interestingly, one study showed that KSHV can transform human umbilical vein endothelial cells (HUVEC) and that the levels of telomerase in these transformed cells were elevated (Flore et al. 1998). Telomerase is a holoenzyme that prevents chromosome degradation, end-to-end chromosome fusions, and chromosome loss and maintains telomere length by the addition of new repeat sequences at the ends of the chromosomes, thus deriving cell immortalization (Greider 1991a, b; Harrington and Greider 1991). In support of these studies LANA has also been shown to upregulate the human telomerase promoter through interaction with Sp1 transcription factor, supporting the role of LANA as an oncoprotein (Knight et al. 2001; Verma et al. 2004). Additional evidence suggests that LANA cooperates with HIV Tat and upregulates the HIV LTR promoter, thus supporting the hypothesis that KSHV coinfection promotes HIV propagation (Hyun et al. 2001; Knight et al. 2001). Furthermore, LANA can also activate the major latent EBV promoter elements (LMP1 and Cp1) in transient studies (Groves et al. 2001).

KSHV prevents the cell surface expression of MHC chains and protects itself from CTL cell lysis during the lytic cell cycle. However, as only a subset of the infected cells undergo lytic replication (1%–5%), KSHV is likely to have a means to protect itself from the immune response during latent infection (Staskus et al. 1997; Zhong and Ganem 1997; Dittmer et al. 1998; Sarid et al. 1998; Katano et al. 1999; Talbot et al. 1999; Parravicini et al. 2000). Infection of primary dermal microvascular endothelial cells with KSHV resulted in downregulation of cell surface MHC class I, CD31 (PE-CAM), and CD54 (ICAM-I) immune response modulators. Moreover, this downregulation was correlated with increased levels of LANA, suggesting that during latency LANA may have a role in the downregulation of MHC class I molecules (Tomescu et al. 2003).

More recent, interesting studies have demonstrated a role for LANA in regulating KSHV reactivation, and this involves modulation of the major Notch cellular signaling pathway (Lan et al. 2005b). In our most recent studies, we demonstrated that intracellular activated domain of Notch1 (ICN) is accumulated in KSHV latently infected cells through stabilization mediated by LANA (Lan et al., unpublished data). This increase in levels of ICN is crucial for oncogenesis induced by KSHV as shown by the significant increase in proliferation of KSHV-infected cells. The ability of ICN to potentially reactivate the virus through upregulation of RTA can be effectively antagonized by KSHV LANA. This provides a finely tuned model of virus-host interaction in which KSHV selectively usurps the function of a conserved cellular signaling pathway to achieve oncogenesis while dispensing additional function to strictly maintain latency. Taken together, these studies now clearly show a feedback mechanism whereby KSHV can establish latency by repressing the expression of its major transactivator. Further work is needed to explore this new functional role of LANA. However, so far we can say with confidence that LANA plays a critical role in KSHV-mediated tumorigenesis by regulating transcription, chromatin remodeling, episomal maintenance, replication of genome, and control of reactivation and thus maintenance of KSHV latency.

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Fig. 1.

Schematic diagram of the major KSHV latency cassette. Location of the major latency transcripts cluster in KSHV genome is shown. *Numbers* indicate nucleotide positions, based on the database sequence NC_003409 (Russo et al. 1996). *Black boldface arrows* represent ORF71, 72, and 73; *gray boldface arrow* represents ORF74

Fig. 2. LANA shows punctuate nuclear staining. Immunofluorescence analysis showed that LANA was localized to nucleus of the different KSHV latently infected B lymphoma cell lines BCBL1, BC3, and JSC

Fig. 3.

Scheme showing the structure and functional domains of LANA. LANA is a 1162-amino acid protein nuclear protein (BC-1 strain). Numbers indicate the amino acids (*aa*). The putative domains include an N-terminal proline-rich domain (*P-rich*); aspartic acid, glutamic acid repeat region (*DE*); glutamine-rich domain (*Q-rich*); leucine zipper (*LZ*); LANA also has two nuclear localization signals (*NLS*) in both the N- and C-terminal regions. The approximate position of each functional domain is shown by a *black bar*, corresponding to the function shown in the column on the left (Cotter and Robertson 1999; Friborg et al. 1999; Platt et al. 1999; Krithivas et al. 2000; Schwam et al. 2000; Cotter et al. 2001; Groves et al. 2001; Hyun et al. 2001; Lim et al. 2001; Krithivas et al. 2002; Mattsson et al. 2002; Shinohara et al. 2002; Borah et al. 2004; Lan et al. 2004, 2005a)

Fig. 4.

Fig. 4A–C Hypothetical model to show interplay between LANA and RTA. **A** During the latent infection of KSHV, LANA represses RTA expression through physical interaction with RBP-Jκ. **B** On reactivation of the virus, RTA can autoactivate its own expression then initiate the cascade of lytic gene expression. **C** In early stage of primary infection, RTA can activate LANA expression through functional association with RBP-Jκ

Fig. 5. Model showing modulation of Wnt signaling pathways through interaction with LANA. LANA binds to GSK-3 β and translocates this to the nucleus, leading to the depletion of cytoplasmic GSK-3 β . This results in accumulation of unphosphorylated β -catenin, which in turn translocates to the nucleus and associates with the LEF and TCF family of transcription factors, thus modulating gene expression controlled by these transcription factors

Fig.6.

Modulation of pRb and p53 pathways in KSHV-infected cells. Latency-associated nuclear antigen (LANA) competes with E2F transcription factor for binding to pRb and releases the E2F transcription, which leads to the upregulation of E2F-modulated genes. LANA also interacts with p53 and blocks the p53-mediated apoptosis, thus helping in cell cycle progression and tumorigenesis

Fig. 7.

Fig. 7A, B pCBP/p300. **A** Schematic representation of domains involved in binding to various cellular and viral proteins. LANA interacts with C/H3 domain of the protein, which is also the binding region of other viral encoded proteins including HPV E6, E1A SV40 LT, EBNA3C. **B** Downregulation of CBP leads to the upregulation of c-myc and cyclin D1 and thus induction of tumorigenesis

Fig. 8.

Model of KSHV episomes tethering in KSHV-infected cells. C terminus of LANA binds to its cognate sequence in TR region of KSHV genome. N terminus of LANA attaches it to nucleosome with the help of cellular proteins including histone H1, heterochromatin protein 1 (HP-1). Cellular proteins MeCp2 and DEK help in tethering of the viral genome to the host chromosome