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Current Opinion in Virology

Kaposi sarcoma-associated herpesvirus latency-associated nuclear antigen: more than a key mediator of viral persistence

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Kaposi sarcoma-associated herpesvirus (KSHV), or human herpesvirus-8, is an oncogenic herpesvirus. Its latency-associated nuclear antigen (LANA) is essential for the persistence of KSHV in latently infected cells. LANA mediates replication of the latent viral genome during the S phase of a dividing cell and partitions episomes to daughter cells by attaching them to mitotic chromosomes. It also mediates the establishment of latency in newly infected cells through epigenetic mechanisms and suppresses the activation of the productive replication cycle. Furthermore, LANA promotes the proliferation of infected cell by acting as a transcriptional regulator and by modulating the cellular proteome through the recruitment of several cellular ubiquitin ligases. Finally, LANA interferes with the innate and adaptive immune system to facilitate the immune escape of infected cells.

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Current Opinion in Virology 2023, **61**:101336

This review comes from a themed issue on **Chronic Infections**

Edited by **Thomas Mertens**

Available online xxxx

https://doi.org/10.1016/j.coviro.2023.101336

Introduction

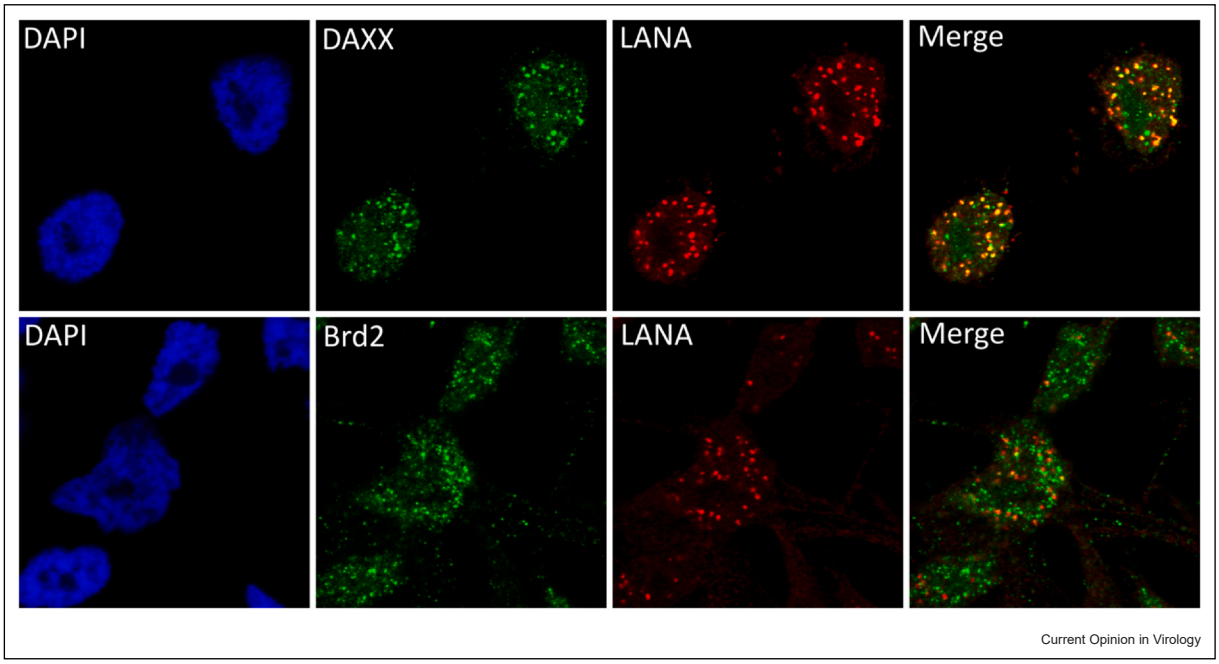
Kaposi sarcoma-associated herpesvirus (KSHV) is the infectious cause of Kaposi sarcoma (KS), primary effusion lymphoma (PEL), and the plasma cell variant of multicentric Castleman's disease (MCD) and has been classified by the International Agency for Research against Cancer as a human class-I carcinogen [1]. KSHV can persist in infected individuals, most of whom will never suffer clinical consequences. Rarely, however, infection with KSHV can lead, at an older age and more commonly in men, to the development of a slowly progressing form of Kaposi sarcoma referred to as 'classic' KS, first described by M. Kaposi in 1872 (for a previous review, see Ref. [2]). The likelihood of being infected with or reactivating KSHV, and with it the risk of developing KS, is increased by other infections, as, for example, helminth infections and malaria in East and Central Africa [3,4], or HIV in the case of the AIDS-associated form of KS (for a previous review, see Ref. [2]).

The ability of KSHV to persist in infected individuals is considered a prerequisite for the development of KSHV-associated tumors. The viral latency-associated nuclear antigen (LANA) plays an essential role during the establishment and maintenance of viral persistence and latency, controls reactivation from latency, and contributes to tumorigenesis and the immune escape of KSHV-infected cells. In this review, we focus on how our understanding of this viral protein has evolved over the last decade and refer the reader to several reviews that cover the earlier literature [2,5–7].

LANA domains and structure

The term 'latency-associated nuclear antigen' (abbreviated LANA or LNA) originally referred to a 'speckled' nuclear immunofluorescence staining pattern seen in KSHV-infected PEL cells labeled with KS patient sera (Figure 1) [2,7]. The viral gene encoding LANA, open-reading frame (ORF) 73, was identified by screening phage cDNA expression libraries prepared from PEL cell lines with KS patient sera ([8]; further references in [7]). As shown in Figure 2, LANA consists of three main regions: an aminoterminal domain (N-terminal domain (NTD)), a central domain consisting of a variable number of multiple repeated elements (internal repeat region (IR)), and a carboxyterminal domain (CTD) that contains the binding domain for the latent KSHV origin (DNA-binding domain (DBD)). Owing to the variable number of repeat elements in the central domain, the overall length of LANA differs between KSHV isolates, with the prototypic LANA sequence (BC-1 strain) consisting of 1162 amino acids [9].

Figure 1



Immunofluorescence image of the typical LANA-containing nuclear bodies ('speckles'). The PEL cell line BCBL1 was stained with an antibody to LANA (IR, see text and Figure 2), followed by a rhodamine X-coupled second antibody. Two cellular proteins interacting with LANA, DAXX and Brd2 (see text and Figure 2), were stained with specific antibodies, followed by a fluorescein isothiocyanate-conjugated second antibody and are shown to colocalize with LANA.

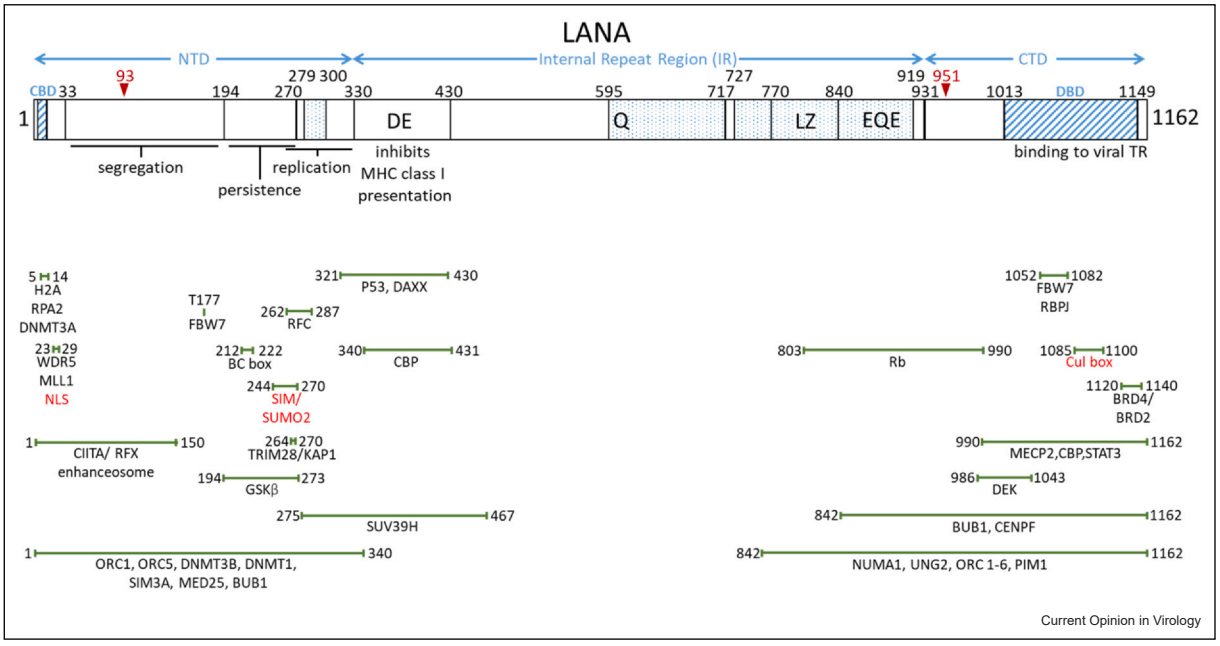
The NTD binds to mitotic chromosomes by virtue of a short chromatin-binding site (CBD, aa 5-14, Figure 2) that interacts with the basic surface of histone 2A/B (H2A and H2B) [10], phosphorylated H2AX [11], as well as the DNA methyl transferase DNMT3a and the replication proteins RPA2 and SMARCAL1 (references in [2,6,7] and unpublished observation) (Figure 2). Phosphorylation of serine 10 and threonine 14 in this CBD motif by the cellular Ribosomal S6 kinase promotes the interaction of LANA with H2B [12]. The NTD contains a nuclear localization signal (NLS) (aa 24-30), which overlaps with a binding site (aa 23-29) for the WD repeat domain 5 (WDR5) subunit of the histone H3K4 methyl transferase KMT2A/MLL1 complex [13]. Other NTD regions are required for episome segregation (aa 33-194) and episome persistence (aa 194-270) (Figure 2) [14]. The latter overlaps with a region (aa 262-320) required for replication and the site responsible for recruiting cellular replication factor C (RFC, aa 262-287), but also a motif (aa 212-222) recruiting the elongin-B/C component of a ubiquitin E3 ligase complex, a SUMO-interacting motif (SIM, aa244-270), a binding site for KAP1 (aa 264-270), and contains several glycogen synthase kinase-3 beta (GSKß) phosphorylation sites (aa 219, 250, 261, and 268) [15–18], further references in [2,6,7]. The SIM motif recruits several cellular SUMOylated proteins, including KAP1/TRIM28, a transcriptional repressor involved in the regulation of the KSHV lytic cycle [15,19,20].

The first 150 amino acids of the NTD also mediate the interaction of LANA with components of the major histocompatibility complex (MHC) class-II transactivator (CIITA) enhanceosome and thereby the LANA-dependent downregulation of MHC class-II molecules [21].

The internal repeat region of LANA is essential for viral latency and persistence, in cell culture and in mice, as shown by experiments that deleted this region either partially or completely from the KSHV genome or from a recombinant MHV68 carrying KSHV LANA or LANA mutants [22–25]. An acidic DE-rich repeat region (aa 330-421, Figure 2) binds to non-acetylated tumor protein p53 (p53), similar to an acidic reader of clusters of non-acetylated lysine residues on p53, and is required for efficient latent persistence [24]. The same region also inhibits the presentation of LANA-derived peptides on MHC class-I molecules [26]. The binding of the chromatin protein death domain-associated protein (DAXX), which strongly colocalizes with LANA nuclear bodies ('speckles'), has also been mapped to this region [27,28].

Within the CTD, the DBD of LANA binds to the KSHV latent origin of replication, which is located in the

Figure 2



Schematic diagram of LANA and its interaction sites with selected cellular proteins The diagram shows the three main domains of LANA, the N-terminal domain (NTD), the internal repeat region (IR) and the C-terminal domain (CTD), which contains the binding domain (DBD) for three specific viral LANA binding sites (LBS) in the terminal repeat (TR) region of the viral genome. The position of regions or motifs that are important for the ability of LANA to bind to viral DNA and its role as a mediator of viral persistence, latent replication and segregation, as well as the binding sites for selected cellular LANA-interacting proteins, are shown. The molecular structure of the cross-hatched DBD and of short LANA peptides in complex with their ligands has been solved by X-ray crystallography [29-31]. An a-helical structure (stippled marking) has been predicted for a short region in the NTD (aa 279-300) and two regions in the internal repeat region (aa 595-717; 727-919), the second of which contains a leucine zipper (LZ). DE: region rich in aspartate and glutamic acid; Q: region rich in glutamine residues; EQE: region rich in glutamic acid and glutamine residues. Binding sites for LANA-interacting proteins listed in this figure are represented by horizontal green lines. These LANA-interacting proteins include: BUB1 (BUB1 mitotic checkpoint serine/threonine kinase), BRD2/4 (bromodomain containing 2, 4), CBP (CREB binding protein), CENPF (centromere protein F), CIITA (class II transactivator), DAXX (death domain associated protein), DEK (DEK proto-oncogene), DNMT1 (DNA methyltransferase 1), DNMT3A (DNA methyltransferase 3 alpha), DNMT3B (DNA methyltransferase 3 beta), FBXW7 (F-box and WD repeat domain containing 7), GSKß (glycogen synthase kinase-3 beta), H2A (histone 2A/B), MECP2 (methyl-CpG binding protein 2), MED25 (mediator complex subunit 25), MLL1/KMT2A (lysine methyltransferase 2A), NUMA1 (nuclear mitotic apparatus protein 1), ORC 1-6 (origin recognition complex subunits 1-6), p53 (tumor protein p53), PIM1 (Pim-1 proto-oncogene), RB (RB transcriptional corepressor), RBPJ (recombination signal binding protein for immunoglobulin kappa J region), RFC (replication factor C), RFX (regulatory factor X), RPA2 (replication protein A2), STAT3 (signal transducer and activator of transcription 3), SUV39H1 (histone lysine methyltransferase), UNG2 (uracil DNA glycosylase), WDR5 (WD repeat domain 5). Other motifs in LANA, such as NLS (nuclear localization signal), SIM (SUMO-interacting motif), cul box (cullin-binding site) and the two caspase cleavage sites at position 93 and 951 are labelled in red.

terminal repeat (TR) subunits of the KSHV genome (Figure 2). The structure of the LANA DBD, alone and in complex with oligonucleotides derived from the latent origin of replication, shows that the LANA DBD forms dimers and that one DBD dimer binds to each of three LANA-binding sites (LANA-BS) found in every terminal repeat subunit in the viral genome [29–31]. The three individual LANA DBD dimers positioned on one TR subunit can multimerize and thereby bend the latent viral DNA at an angle of approx. 60° [30,32,33]. Furthermore, the LANA CTD interacts with a number of cellular chromatin components, such as the epigenetic 'readers' bromodomain-containing 2, 4 (BRD2 and BRD4), methyl–CpG-binding protein 2 MECP2 recognizing methylated CpGs, components of several E3 ligase complexes [16,34], and transcriptional regulators such as recombination signal-binding protein for immunoglobulin kappa-J region (RBPJ) [35], as reviewed previously [2,6,7] and discussed below.

The assembly of three LANA dimers on each copy of a viral terminal repeat (TR) subunit, the presence of multiple TR subunits in the terminal repeat region of the viral genome, the oligomerization of LANA DBD dimers, and the ability of the LANA NTD to bind to histones H2A/B on chromatinized viral episomes together provide the basis for the formation of the typical LANA nuclear bodies or 'speckles' (Figure 1). These colocalize with cellular proteins such as DAXX, BRD2/4, and ORCs and are required for viral genome persistence and stability (Figure 1) [30,31,36,37]. Only the LANA DBD, parts of the LANA NTD, and two sections of the IR domain have been shown experimentally [10,13,29–31] or predicted (www.bosse-lab.

org) to adopt a defined structure, while most of LANA appears to be intrinsically unstructured. Liquid–liquid-phase separation, that is, the formation of large membraneless organelles also observed for other unstructured proteins, has been suggested to contribute to the formation of LANA nuclear bodies [38].

Alternative forms of LANA

In addition to the canonical, full-length, LANA protein, alternative LANA forms arising from internal translation initiation, premature termination, or internal frameshifting have been reported [39,40]. Internal translation initiation at noncanonical start codons generates LANA variants that lack the aminoterminal nuclear localization signal (NLS) (Figure 2) and are therefore located in the cytoplasm [40]. Such cytoplasmic LANA isoforms have been observed in KSHV-infected cells undergoing lytic replication [28]. Full-length LANA has also been reported to exist in the cytoplasm of infected cells [41]. Internal frameshifting resulting from a ribosomal 2 nucleotide slippage (-2 frameshift) within the LANA internal repeat region (between aa 438 and 769, Figure 2) also leads to formation of a smaller LANA isoform, whose aminoterminal region is identical to canonical LANA, but whose carboxyterminal region results from the translation of a different reading frame, this alternative isoform has been observed in some, but not all PEL cell lines [39]. LANA has also been shown to be proteolytically cleaved by caspases 1 and 3 at two sites located in the NTD and CTD (Figure 2), potentially yielding smaller LANA fragments [42].

LANA mediates the replication and maintenance of latent viral DNA

LANA mediates the replication and partitioning of latent KSHV episomes in dividing cells [43], KSHV mutants with a deleted ORF73 fail to establish persistent infections in tissue culture [22], the reduction of LANA expression in PEL cells by shRNA or HSP90 inhibitors [44] decreases PEL cell proliferation, and the cell-specific knockout of MHV68 LANA interferes with latency establishment in germinal center B cells in MHV68-infected mice [45]; further references in [2,6,7]. To replicate the latent viral episomes during the S phase of dividing, latently infected cells, LANA recruits components of the cellular DNA replication machinery, including several components of the mini chromosome maintenance complex (MCM3,4,6), origin recognition complex subunits 1–6 (ORC1–6), RFC, topoisomerase-II beta, structure-specific recognition protein 1 (SSRP1), proliferating cell nuclear antigen (PCNA), N-myc downstream-regulated gene 1 (NDRG1), nucleosome assembly protein-1-like protein 1 (NAP1L1), and RPA [46–52]; further references in [2,6,7]. PCNA is loaded onto the KSHV TR region by LANA through an indirect interaction with either RFC, which binds to LANA at a site (aa 262–287) in the LANA NTD [50], or the mitotic checkpoint kinase BUB1 [51]. Similarly, NDRG1 also serves as a scaffold protein bridging LANA and PCNA, and NDRG1 knockdown decreases the viral genome copy number in infected cells [52]. LANA protects several DNA replication-associated proteins (CDK2, CCNE, ORC2, MCM3, CDT1, CDC6, and CDC45) from hypoxia-mediated degradation, thus promoting latent KSHV replication under hypoxic conditions [53]. NAP1L1 likely plays a role in the formation of nucleosomes containing newly replicated viral DNA and is therefore also required for their persistence [47].

LANA and cellular and viral gene regulation

LANA can modulate the expression of many viral and cellular genes by regulating the expression of, or interacting with, transcription factors such as JUN [54], HIF-1α [55], RBPJ [35], cAMP response element-binding protein (CBP), MECP2 [56], signal transducer and activator of transcription 3 (STAT3) [57], mediator complex [58], helix–loop–helix protein ID-1 [59], SP1 [60], regulatory factor X (RFX) [21], KRAB zinc finger-interacting protein [61], KAP1 [18], SMAD1 [62], and also repressive factors such as DAXX [63]; further references in [2,6,7].

LANA is deposited near the transcriptional start sites of several cellular and viral promoters that are frequently characterized by epigenetic modifications such as histone acetylation and H3K4 trimethylation, indicative of transcriptionally active chromatin [64–68]. Examples of KSHV promoters occupied by LANA include its own promoter, the promoter of ORF50/(RTA) Replication and Transcription Activator (the master regulator of the lytic replication cycle), as well as several other early or late viral promoters and, most prominently, the viral terminal repeat [64,66,67]. This involves the binding of LANA to DNA sequences with some homology to the three LANA-binding sites (LBS) in the viral terminal repeat subunits, or to other potential LANA-binding motifs [64,65,68]. In addition, LANA is recruited to cellular or viral transcriptional start sites by chromatin components such as the H3K4me3 histone methyltransferase hSET1, DEK proto-oncogene (DEK), MECP2, or BRD2/4, 'readers' of acetylated histones [7,66–68]. Deposition of LANA near several cellular and viral transcriptional start sites is inhibited by BRD4 inhibitors, which also reduce the binding of the cohesion subunit RAD21 and of CCCTC-binding factor to sites in the LANA and lytic promoter regions of the KSHV genome and thereby alter the cohesion-mediated connections between the latent and lytic control regions and promote lytic reactivation [66,69]. While being thus found on several promoters of transcriptionally active cellular genes, LANA only appears to regulate the expression of a small subset of these genes [66–68] and the

functional consequence of this deposition of LANA near cellular and viral transcriptional start sites is therefore not entirely clear. Among the LANA-occupied and also -regulated cellular genes are interferon-γ-regulated genes involved in antigen presentation [67] and SENP6, involved in the regulation of latency [65]. Activation of productive (lytic) KSHV replication leads to the disengagement of LANA from several cellular promoters [68].

The BRD2/4-dependent deposition of LANA near cellular transcriptional start sites is reminiscent of the distribution of gammaretroviral (e.g. Moloney Leukemia virus (MLV)) and piggyback transposon integration sites, which is determined by the interaction of the gammaretroviral integrase, or the piggyback transposase, with BRD family members [70,71]. Similarly, the human papillomavirus latent origin-binding protein E2 also binds to BRD family members to locate viral genomes near fragile sites in the cellular chromatin [72,73]. KSHV LANA, and also mLANA, the LANA homolog of the related gammaherpesvirus MHV68, may therefore utilize a similar mechanism to associate itself with certain cellular promoters and enhancers, or particular chromatin regions [66]. Chromodomain helicase-binding protein 4 (CHD4), activity-dependent neuroprotective protein (ANP), and heterochromatin protein 1 (HP1γ) form the ChAHP complex, interact with LANA, and have been shown to locate LANA and latent viral DNA to particular sites in the cellular genome; CHD4 represses the productive (lytic) replication cycle and is targeted by the KSHV PAN RNA, which is required for lytic reactivation cycle and may act by removing CHD4 from viral lytic promoters [74,75].

LANA and epigenetic modifications of viral or cellular DNA

The deposition of histones on the viral genome and their epigenetic modification occurs after entry of the virus into the cell and upon circularization of the viral genome [76–79]. This is followed, with some delay, by the methylation of the viral genome [76]. Establishment of KSHV latency is associated with deposition of H3K27me3, a bivalent mark representing 'poised' heterochromatin that can repress transcription, despite the presence of activating modifications, on the promoters of lytic genes [76]. LANA recruits several chromatin-modifying cellular proteins, including polycomb-repressive complex 2 (PRC2), which contains the H3K27me3 histone methyltransferase EZH2, the H3K9 methyltransferase SUV39H, the H3K4 methyltransferase SET1, a H3K9 demethylase, the *de novo* DNA methylase DNMT3A, the histone acetyltransferase CBP, the histone deacetylase SIN3, and chromatin remodelers (FACT, CBP, and BRD proteins) [64,77,79,80]; further references in [6,7]. A role for LANA in the epigenetic modification of the KSHV or cellular genome, in particular in the deposition of the H3K27 trimethylation mark, has therefore been suggested [79] and LANA also upregulates the expression of the H3K27me3 histone methyltransferase EZH2 [81]. In addition, cis-acting elements in the KSHV genome, such as its high CG content, may determine why KSHV, unlike many other herpesviruses, is rapidly silenced by the acquisition of repressive heterochromatin on its latent viral genome [82]. The high CG content of the KSHV has been suggested to facilitate the LANA-independent recognition of incoming (non-chromatinized) viral genomes by the PRC1.1 complex through the recruitment of its KDM2B subunit to unmethylated CpG motifs; KDM2B, a histone demethylase, removes activating histone marks H3K4me3, H3K26me2, and H3K79me2, which leads to the ubiquitination of H2A lysine 119 (H2A–K119ub) and the subsequent recruitment of PRC2 and deposition of the repressive H3K27me3 mark [75,82].

LANA as a regulator of lytic viral replication

As deduced from many studies using latently infected cell lines in which the productive (lytic) KSHV replication cycle has been induced by treatment with Tumor-promoting agent, Na butyrate, and other histone deacetylase inhibitors, or hypoxia, LANA plays an important role in repressing the productive replication cycle and thereby ensures latent viral persistence. The KSHV lytic replication cycle is initiated by RTA, a transcriptional activator of many lytic viral genes. The promoter of the viral gene encoding RTA, ORF50, and also the promoters of several other early viral genes, contain binding sites for cellular transcription factors such as RBPJ, HIF-1α, AP-1, SP1, OCT1, nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NFkB), and NRF2; these mediate the activation of the ORF50 promoter by RTA itself, hypoxia, Mitogen-activated protein kinase (MAP) kinases, or inhibitors of histone deacetylases. LANA represses the ORF50 promoter by binding to RBPJ and/or SP1 [35,83,84]. Neddylation of LANA may be involved in its interaction with RBPJ and the LANA-mediated repression of the ORF50 promoter [85], while the increased acetylation of LANA, induced by histone deacetylase inhibitors such as Na butyrate, disrupts its interaction with SP1 and reduces its ability to bind to the ORF50 promoter and to repress lytic replication [84]. Repression of the ORF50 promoter by LANA also involves the LANA-mediated recruitment of the repressor TRIM28/KAP1 [15,18]. LANA may bind to TRIM28 either directly [18] or by virtue of its SIM (SUMO-interacting motif), which recognizes sumoylated TRIM28 [15]. The LANA–TRIM28 complex has also been shown to include RBPJ [20] and the transcription factor NRF2 [86]. Silencing of TRIM28 facilitates hypoxia-induced activation of several KSHV lytic genes and promotes the loss of KSHV episomes [20].

In contrast to these studies with latently infected cell lines, KSHV has been shown to spontaneously undergo productive replication in infected primary lymphatic endothelial cells (LECs) [87–90]. Deleting the gene encoding LANA from the viral genome reduces viral lytic replication in LECs [90]. LANA has been reported to dampen innate immune responses, such as the induction of interferon signaling induced by Cyclic GMP-AMP synthasec (cGAS) and the activation of NFkB triggered by the MRN complex (MRN) DNA repair complex, in order to promote KSHV productive replication [28,91].

LANA recruits ubiquitin E3 ligases and sumoylated proteins

As discussed above, a SIM in the LANA NTD has been reported to recruit a number of cellular proteins, including the transcriptional repressor TRIM28/KAP1 [15,19,92]. LANA has also been reported to assemble a ubiquitin E3 ligase complex by virtue of a bipartite SOCS box, which allows the recruitment of elongin B/C to a motif in the LANA NTD (elongin BC box, aa 212-222) and of cullin-5 to a cullin box in the LANA C-terminal DBD (aa 1085-1100); this complex could ubiquitinate cellular targets such as p53, von Hippel-Lindau tumor suppressor (VHL), or NFkB p65 [16,93] and degradation of VHL may be linked to the increased expression of HIF-1α seen in KSHV-infected cells [55]. The mLANA protein of murine gammaherpesvirus-68 can also assemble an elongin B/C–cullin-5 E3 ligase targeting NFkB and c-MYC [94]. Mutations in the mLANA DBD that interfere with the assembly of the E3 ligase complex allow episomal persistence in tissue culture but negatively affect the expansion of MHV68-infected germinal center B cells in mice, suggesting that the ability of mLANA to assemble an elongin B/C–cullin-5-containing E3 ligase complex may be important for viral persistence in B cells *in vivo* [95].

LANA has furthermore been reported to recruit three other ubiquitin E3 ligases or their components, RLIM, FBW7, and the APC/C complex, and may thereby enhance or decrease the ubiquitination and proteasomal turnover of their physiological cellular protein targets [34,96–98]. This includes the reported proteasomal degradation of the mitotic checkpoint kinase BUB1, an APC/C target, potentially leading to chromosomal instability [97]. On the other hand, by removing RLIM and FBW7 from their targets, LANA may protect the RLIM target TRF1, as well as the FBW7 targets MCL-1 and intracellular notch (ICN), from proteasomal degradation [34,96,98]. Stabilization of MCL-1 and ICN by LANA may promote the proliferation and/or apoptosis of KSHV-infected PEL cells [34,96]. The recruitment of FBW7 by LANA has been reported to involve either a phosphodegron motif (T177) in the LANA NTD [96], or a region in the LANA C-terminal DBD (aa 1052-1083) [34]. Similarly, LANA may prevent the proteasomal degradation of the notch target HEY1 and thereby promote angiogenesis [99]. LANA also interacts with, and inhibits the expression of, the SUMO-specific peptidase SENP6, which can reduce SUMOylation of LANA and this may contribute to the regulation of latency [65]. Furthermore, LANA has been reported to stabilize PAR3 and to thereby increase the levels of SNAIL, both regulators of epithelial–mesenchymal transition (EMT), in KSHV-infected B cells [100].

LANA and cell cycle checkpoints

LANA can promote cell proliferation. Overexpression of LANA in transgenic mice leads to splenic follicular hyperplasia [101] and LANA can transform primary rodent fibroblasts when cotransfected with HRas (reviewed in Ref. [2]). To achieve these effects, LANA bypasses several cell cycle checkpoints, as recently reviewed elsewhere [102]. Prominent examples include the ability of LANA to bind to, ubiquitinate, increase the degradation, and thereby antagonize the function of the tumor suppressor p53 [16], the related p73 protein [103], and pRB, thereby antagonizing the G1/S checkpoint (references in [102]). LANA directly interacts with CHK2 to antagonize the ATM-/ATR-mediated G2/M checkpoint arrest [104]. LANA has been reported to bind to and promote the proteasomal degradation of the mitotic checkpoint kinase BUB1 and to thereby inhibit the BUB1-dependent phosphorylation of H2A, leading to chromosomal instability [97,105].

LANA and immune regulation

In comparison with some other herpesviruses, KSHV appears to induce only a weak T-cell response against few viral proteins, at least as measured by the stimulation of IFNγ-producing T cells with overlapping peptides covering all KSHV ORFs [106]. The DE-rich element in the LANA internal repeat region (aa 321-430, see Figure 2) inhibits MHC class-I peptide presentation by retarding their processing prior to the translocation of cytoplasmic peptides into the endoplasmic reticulum and loading onto MHC class-I molecules [26]. LANA also inhibits the expression of MHC class-II molecules by two related mechanisms: it binds to IRF4 and thereby inhibits the IRF4-dependent expression of the MHC CIITA [107] and it disrupts the association of CIITA with the MHC class-II enhanceosome by binding to components of the RFX complex [21].

LANA also interferes with two innate immune sensors of cytoplasmic DNA, cGAS and the cytoplasmic MRN complex, and their downstream activation of interferon and NFkB signaling [28,91]. LANA promotes nuclear NFkB p65 ubiquitination and degradation and inhibits

NFkB-dependent IL8 expression by virtue of its ability to assemble an E3 ligase complex consisting of LANA, elongin B/C, cullin-5, and p65 [93].

LANA and LANA-dependent molecular mechanisms as a therapeutic target

In view of the central role of LANA during KSHV persistence, several attempts have been made to develop small-molecule inhibitors directed at LANA [108–110], or to repurpose existing drugs for inhibiting LANA or cellular proteins associated with, and essential for, LANA functions [44,96,111,112]. The former include newly developed compounds that bind to LANA and inhibit its ability to interact with the latent viral origin of replication in *in vitro* assays, but none of these have yet been reported to interfere with KSHV latent persistence or the survival of latently infected cells [108–110]. However, mubritinib, a receptor tyrosine kinase inhibitor, has been shown to inhibit the binding of LANA to the KSHV terminal repeat, to inhibit LANA-dependent transcriptional activation, but also acts as an electron transport inhibitor; these pleiotropic effects may contribute to its ability to inhibit the growth of PEL cell lines in tissue culture and in a mouse model [111].

Among other repurposed compounds, HSP90 inhibitors such as AUY922 promote the proteasomal degradation of LANA and inhibit the growth of KSHV-infected cells in a xenograft model of KS [44]. Cytarabine inhibited the synthesis of viral DNA and RNA, induced LANA degradation, and inhibited the growth of PEL tumors in a xenograft mouse model [112]. LANA stabilizes levels of the cellular anti-apoptotic protein MCL-1 by binding to and removing the E3 ligase FBW7 from MCL-1, and MCL-1 inhibitors suppress the the growth of PEL tumors in a xenograft model [96].

LANA forms a complex with p53 and its ubiquitin E3 ligase MDM2 and this complex can be disrupted with the MDM2 inhibitor nutlin 3A; in PEL cells without p53 mutations, this restores p53-dependent DNA-damage signaling and apoptosis [113]. LANA also binds to and stabilizes the p53 homolog p73; the small molecule compound RETRA disrupts this complex and induces apoptosis in PEL cells with a mutated p53 [103]. Thus, nutlin 3A and RETRA can restore p53 and p73 function in PEL cells with wt or mutated p53, and could represent candidates for the treatment of this KSHV-associated lymphoma.

Conclusion

Over the last decade, significant progress has been achieved in our understanding of how KSHV LANA, the key mediator of viral persistence and regulator of latency, functions at the molecular level. This has provided the basis for ongoing efforts to target LANA, or LANA-dependent cellular processes, with small molecule inhibitors in an attempt to eliminate latently infected cells and to inhibit the growth of KSHV-infected tumor cells in KS, PEL, or MCD.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

None.

Acknowledgements

Work in the TFS laboratory is supported by German Research Foundation (DFG) through the Excellence Cluster RESIST (EXC 2155) and the Collaborative Research Center (SFB/CRC) 900, project C1, as well as by the German Center for Infection Research (DZIF), project 07.829.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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