

THE KSHV LATENCY-ASSOCIATED NUCLEAR ANTIGEN: A MULTIFUNCTIONAL PROTEIN

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1. ABSTRACT

Kaposi's sarcoma(KS)-associated herpes virus (KSHV) or human herpesvirus 8 (HHV-8) is highly associated with KS, primary effusion lymphoma (PEL), and multicentric Castleman's disease, an aggressive lymphoproliferative disorder (1-3). Most tumor cells are latently infected with KSHV in which a small subset of viral genes are expressed (4-6). Of these latently expressed genes, the latency-associated nuclear antigen (LANA1, LNA, or LNA1) is the only protein consistently shown to be highly expressed by in situ hybridization and immunohistochemistry (7-10). In the past few years multiple functions have been demonstrated for LANA1. Here we review LANA1's roles in KSHV infection. Topics discussed include LANA1's roles in episome persistence, regulation of transcription and interaction with cellular proteins.

2. LANA1 AND EPIDEMIOLOGY

LANA1 was initially detected as punctate nuclear staining by indirect immunofluorescence microscopy performed on KSHV infected PEL cell lines using serum from KSHV infected individuals (8, 11). Subsequently, base-line anti-LANA1 reactivity was used as a marker for KSHV infection (8, 12). Work with the anti-LANA1 assay in a clinic-based population found an HHV-8 seroprevalence of 27 percent in homosexual or bisexual

men (8). Similar estimates have been obtained with other serologic assays that measure antibodies to lytic-phase viral antigens (11, 13). After the KSHV genome was sequenced, LANA1 was mapped to KSHV ORF73 (9, 10, 14).

3. TRANSCRIPTION AND SEQUENCE

LANA1 is expressed from a polycistronic message which also contains ORF 71 (v-FLIP) and ORF72 (v-cyclin), both of which are downstream of ORF73 (10, 15, 16). The LANA1 message is spliced once upstream of ORF73, although an unspliced message has also been described (15). Potential transcription factor binding sites for SP-1, IRF 1, IRF 2 and c-myc are upstream of the transcription initiation site (15, 16). Reporter assays in 293 and BJAB cells showed robust activation using the ORF 73 promoter (15, 16). ORF 73 promoter sequence was furthermore subject to cell cycle regulation, although whether this phenomenon is a result of the presence of v-cyclin on the same transcript remains to be determined (16).

KSHV ORF 73 encodes a protein of variable size in different viral isolates. LANA1 encodes an N-terminal proline rich domain, an internal glutamine rich and acidic repeat domain followed by a leucine zipper motif (Figure 1) (17, 18). The number of repeat elements can vary

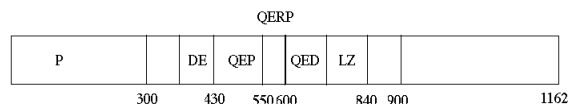


Figure 1. Schematic diagram of LANA1. Numbers denote the amino acids (aa). Proline rich domain (P) (aa 60-260); aspartic acid, glutamic acid repeat region (DE) (aa 330-430); glutamine, glutamic acid and proline rich repeats (QEP) (aa 440-550); glutamine, arginine, glutamic acid and proline rich repeats (QERP) (aa 550-595); glutamine, aspartic acid and glutamic acid rich repeats (QDE) (aa 595-750); leucine zipper (LZ) (aa 770-840).

between KSHV isolates, and accounts for the different LANA1 sizes (19). However, within any one isolate, the number of repeats remains stable, during both lytic and latent infection.

4. LANA1 EXPRESSION IN KS, PEL, AND MULTICASTLEMAN'S DISEASE

LANA1 is expressed in KS, multicentric Castleman's disease, and primary effusion lymphoma (PEL). In KS, LANA1 is expressed in the spindle cells in early patch and plaque stages as well as the advanced nodular stage (7, 10, 20, 21). These results suggest a role for KSHV in the early pathogenesis of KS development. In multicentric Castleman's disease, LANA1 is expressed in the mantle zone of lymph node follicles in large immunoblastic B cells (7). LANA1 is also expressed in primary PEL cells as well as PEL cell lines (7, 22).

5. LANA1 SHARES HOMOLOGY WITH ORF 73'S OF OTHER GAMMA-2 HERPESVIRUSES

LANA1 is homologous to ORF 73s of other gamma-2 herpesviruses (17, 18, 23) but ORF 73's of other gamma-2 herpesviruses vary in the regions of homology that they share with KSHV LANA1. For instance, rhesus rhadinovirus (RRV) ORF 73 (447 aa), (24, 25) and murine herpesvirus 68 (MHV 68) ORF 73 (314 aa) (23) both have an N-terminal proline rich domain similar to LANA1, but this domain is absent in herpesvirus saimiri (HVS) ORF 73 (407 aa) (26). HVS ORF73, unlike RRV or MHV 68 ORF 73, has a repeat region rich in glutamic acid. However all the viral ORF73s share homology at their C-termini which suggests conserved function(s) for this domain.

6. LANA1 SUBCELLULAR LOCALIZATION

Detailed analyses of the subcellular distribution of LANA1 have been performed by confocal microscopy. Three-dimensional computer controlled wide field epifluorescence microscopy demonstrated that LANA1 resides in irregularly shaped bodies which preferentially associate with the border of heterochromatin in BCBL-1 PEL cell nuclei (27). LANA1 does not colocalize with ND10 PML bodies (27). Both the N- and C- terminal LANA1 domains are capable of localizing to the nucleus (28). Recent work shows that a region encompassing amino acids 5 to 22 is sufficient to mediate a specific

interaction of LANA1 with chromosomes during mitosis (29) and that LANA1 nuclear localization also maps to a signal comprising amino acids 24 to 30 (29).

Confocal microscopy demonstrated that LANA1 and KSHV genomes colocalize in PEL cells in interphase nuclei and along chromosomes (30, 31). These findings were consistent with the independent findings that LANA1 (32) and KSHV DNA (1) associate with chromosomes in PEL cells. These results also suggested a role for LANA1 in KSHV episome persistence.

7. LANA1 MEDIATES KSHV EPISOME PERSISTENCE BY ACTING ON TERMINAL REPEAT (TR) DNA

LANA1 is necessary and sufficient for the persistence of KSHV episomes containing a specific cis-acting KSHV sequence (30). We have recently localized the cis-acting sequence to the 0.8 kb KSHV TR unit (33). In KSHV-uninfected cells, a plasmid containing KSHV TR elements persists as an episome in the presence of LANA1 (30). Of note, LANA1 bound *in vitro* to the KSHV Z6 cosmid, which includes the KSHV TR elements (31). More recently, LANA1 was shown to bind to nt 603 to 622 of the KSHV TR (33, 34). High copy number, tandemly repeated TRs likely mediate focal concentration of LANA1 to dots in KSHV infected cells.

Since LANA1 colocalizes with KSHV genomes on chromosomes and mediates episome persistence of KSHV DNA, these data are consistent with the model that LANA1 functions to tether KSHV TR DNA to chromosomes during mitosis in order to mediate efficient segregation to progeny nuclei (30, 31). This model of tethering to mediate efficient persistence has been previously proposed for EBV EBNA1 and the bovine papillomavirus E2 proteins (35-40). EBNA1 mediates EBV episome persistence by acting on a 1.8 kb EBV (oriP) element (36) which contains multiple EBNA1 binding sites (35). The functional homology between LANA1 and EBNA1 exists in the absence of any real sequence homology (30). Further, LANA1 does not colocalize with the Epstein-Barr (EBV) EBNA1 protein in the context of PEL cells coinfecting with KSHV and EBV (32).

8. LANA1 POTENTIAL FOR GENE THERAPY

Adenoviruses, retroviruses, and adeno-associated viruses (AAV) are currently used to deliver genes to tumor cells or to supply a functional gene product in cells lacking one. However, these approaches have potential drawbacks. Retroviral vectors integrate into host chromosomes and therefore are subject to position effect variegation in which gene expression is affected by the integration site (41). Infectious viral vectors may elicit an immune response, creating difficulties in immune competent individuals. Therefore, the potential of plasmid-based expression vectors has led to increasing interest. A plasmid expressing specific genes of interest which contains the cis-acting TR unit and a promoter driving the expression of LANA1

should be maintained extrachromosomally, replicate during the cell cycle, and be efficiently partitioned to daughter cells during mitosis.

9. LANA1 TRANSCRIPTIONAL REGULATION

Multiple LANA1 effects on transcription have been demonstrated. LANA1 repressed the EBV virus-latency promoters Cp and Qp in Hela or Rael cells (42). However, in a different report, LANA1 activated both the EBV LMP1 and C promoters in BJAB cells and 293 cells (43). These different observations may be due to different cell types used in the experiments. LANA1 also modulates NF-kappa B-dependent transcription (43, 44). The internal repeats and the C-terminal domain of LANA1 both bind to the C/H3 region of (CREB)-binding protein (CBP) (45). Many proteins, including NF-kappa B, use CBP either as a co-activator or target it as an integrator of transcriptional regulation (46).

LANA1 domains were fused to the Gal4 DNA-binding domain to investigate LANA1 transcriptional regulation (28). Both the LANA1 N- and C-terminal regions repressed transcription with similar efficiency to the wildtype LANA1 in 293T cells (28). However, in HeLa cells, only the N-terminal regions of LANA1 repressed transcription (42).

A recent report demonstrated that LANA1 transactivates the telomerase reverse transcriptase promoter in 293T, 293, and BJAB cells (47). Telomerase reverse transcriptase is the subunit responsible for the enzymatic activity of telomerase. In addition, five Sp1 sites lay adjacent to the promoter, and experiments show that LANA1 affects the Sp1-DNA complex in the context of BJAB nuclear extracts.

10. LANA1 INTERACTS WITH CELLULAR PROTEINS

Yeast two-hybrid analysis using the LANA1 C-terminus as bait identified RING3 as a LANA1 interacting protein (48). RING3 belongs to the Drosophila female sterile homeotic (fsh) family of proteins (48). Since work has shown that RING3 is a potential mitogen-activated nuclear serine/threonine kinase, its ability to affect LANA1 phosphorylation has been investigated (48). RING3 induces LANA1 phosphorylation on serine and threonine residues in in vitro kinase assays and phosphorylation occurs between LANA1 amino acids 951 to 1107. However, RING3 does not directly phosphorylate LANA1 since a mutation in the RING3 catalytic residues which ablates kinase activity does not reduce LANA1 phosphorylation. Instead, RING3 appears to recruit a kinase which phosphorylates LANA1 (48).

LANA1 also interacts with the tumor suppressor gene product p53 (49). LANA1 bound to p53 in vitro and in co-immunoprecipitation assays from cells. LANA1 also inhibited p53 transactivation in reporter assays. Co-expression of LANA1 reduced p53 mediated apoptosis in SAOS-2 cells and NIH/3T3 cells. LANA1's did not cause p53 degradation or alter p53's ability to bind DNA. The p53-binding domain, and transcriptional repression

activity defined in these studies appears to map outside of the first 440 LANA1 amino acids. Therefore, LANA1 may contribute to oncogenesis by promoting cell survival through alteration of p53 function (49).

LANA1 residues 803-990 interact with the "pocket" region of the retinoblastoma protein (pRb) (50). LANA1 transactivated an artificial promoter carrying the cell cycle transcription factor E2F DNA-binding sequences and also upregulated the cyclin E (CCNE1) promoter, but not the B-myb (MYBL2) promoter (50). LANA1 overcame the RB induced flat cell phenotype in SAOS cells and transformed primary rat embryo fibroblasts together with the cellular oncogene Harvey rat sarcoma viral oncogene homolog (Hras), (50). These findings indicate that LANA1 may contribute to oncogenesis by targeting the retinoblastoma protein-E2F transcriptional regulatory pathway.

11. CONCLUSION

LANA1 has a central role in KSHV biology. LANA1 mediates KSHV episome persistence and has transcriptional regulatory properties. LANA1 also interacts with multiple cell proteins. Future work should further define LANA1's role in tumorigenesis and the molecular mechanisms by which LANA1 functions.

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Abbreviations: KSHV (Kaposi's sarcoma-associated herpes virus); HHV8 (human herpesvirus 8); PEL (primary effusion lymphoma); (PEL), latency-associated nuclear antigen (LANA1, LNA, or LNA1)

Key Words: KSHV (Kaposi's sarcoma-associated herpes virus); HHV8 (human herpesvirus 8); PEL (primary effusion lymphoma); (PEL), latency-associated nuclear antigen (LANA1, LNA, or LNA1), Review

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