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RFHVMn ORF73 is structurally related to the KSHV ORF73 latency-associated nuclear antigen (LANA) and is expressed in retroperitoneal fibromatosis (RF) tumor cells

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Abstract

Retroperitoneal fibromatosis herpesvirus (RFHV), the macaque homolog of the human rhadinovirus, Kaposi's sarcoma-associated herpesvirus (KSHV), was first identified in retroperitoneal fibromatosis (RF) tumor lesions of macaques with simian AIDS. We cloned and sequenced the ORF73 latency-associated nuclear antigen (LANA) of RFHVMn from the pig-tailed macaque. RFHVMn LANA is structurally analogous to KSHV ORF73 LANA and contains an N-terminal serine–proline-rich region, a large internal glutamic acidic-rich repeat region and a conserved C-terminal domain. RFHVMn LANA reacts with monoclonal antibodies specific for a glutamic acid–proline dipeptide motif and a glutamic acid–glutamine-rich motif in the KSHV LANA repeat region. Immunohistochemical and immunofluorescence analysis revealed that RFHVMn LANA is a nuclear antigen which is highly expressed in RF spindloid tumor cells. These data suggest that RFHV LANA is an ortholog of KSHV LANA and will function similarly to maintain viral latency and play a role in tumorigenicity in macaques.

Keywords: LANA; Latency associated; Retroperitoneal fibromatosis; Rhadinovirus; ORF73; KSHV; RFHV; Kaposi's sarcoma

Introduction

Kaposi's sarcoma-associated herpesvirus (KSHV)/human herpesvirus 8 was first discovered in AIDS patients in association with Kaposi's sarcoma (KS) (Chang et al., 1994) and has been linked with other AIDS-related malignancies, such as primary effusion lymphomas (PEL) and multicentric Castleman's disease (Cesarman et al., 1995; Soulier et al., 1995). Analysis of the KSHV genome revealed a strong similarity in sequence and gene organization with herpesvirus saimiri (HVS), the prototype of the *Rhadinovirus* genus of gammaherpesviruses found in the New World squirrel monkey (Russo et al., 1996). KSHV is detected in all epidemiologic forms of KS, including classical KS, endemic KS, and AIDS-associated KS, strongly implicating KSHV as the causative agent of the disease (Verma and Robertson, 2003). Essentially all of the characteristic spindle-shaped tumor cells in KS lesions are latently infected with KSHV (Boshoff et al., 1995; Staskus et al., 1997). KSHV latency is characterized by the expression of a restricted set of viral proteins that are believed to play important roles in the maintenance of the viral genome and in the tumorigenesis process leading to the development of KS (Sarid et al., 1998).

The most prominent protein expressed in cells latently infected with KSHV is the *orf73* gene product, the latency-associated nuclear antigen (LANA) (Kedes et al., 1997; Kellam et al., 1997; Rainbow et al., 1997). KSHV LANA is expressed from a polycistronic transcript with two other latent genes, K13,

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encoding the viral FLICE inhibitory protein (v-FLIP), and *orf72*, encoding the viral cyclin D homolog (v-cycD) (Dittmer et al., 1998; Glenn et al., 1999). LANA is a nuclear protein that functions to ensure the maintenance of the viral genome within the host cell by tethering the viral episomal DNA to host cell chromosomes (Ballestas et al., 1999). LANA also regulates the cellular transcription program in KSHV-infected cells through interaction with a number of cellular proteins, including transcriptional regulators, such as the mSin3-associated corepressor SAP30, Sp1, and ATF4/CREB2, as well as known tumor suppressors, p53 and pRB (Friborg et al., 1999; Krithivas et al., 2000; Radkov et al., 2000). Furthermore, KSHV LANA directly influences the viral transcription program and helps to maintain the latent state of the virus by inhibiting viral replication (Lan et al., 2004).

KSHV LANA is a large polypeptide with three structural domains (Russo et al., 1996). The serine- and proline-rich N-terminal domain contains a nuclear localization signal (NLS) (Piolot et al., 2001), a chromatin-binding motif (CBM) (Wong et al., 2004) and domains responsible for interaction with the transcription regulators, mSin3 complex and Sp1 (Krithivas et al., 2000; Verma et al., 2004). The central domain contains several highly repetitive acidic regions that vary in length and are responsible for the size variation of LANA proteins from different KSHV isolates that can range from 1003 to 1162 amino acids (Gao et al., 1999). The proline-rich C-terminal third domain contains another NLS and is responsible for dimerization of LANA and binding to the terminal repeats (TR) of the viral genomic DNA (Ballestas et al., 1999; Cotter and Robertson, 1999; Komatsu et al., 2004). The C-terminal domain is responsible for interaction with tumor suppressors pRB and p53 (Friborg et al., 1999; Radkov et al., 2000).

Two distinct lineages of rhadinoviruses related to KSHV have been identified in Old World primates. The RV1 rhadinovirus lineage consists of KSHV and its homologs in different non-human primate species, including African green monkeys, drills, gorillas, chimpanzees, and macaques (Greensill et al., 2000; Lacoste et al., 2000a; Lacoste et al., 2000b; Schultz et al., 2000). The macaque RV1 rhadinovirus, retroperitoneal fibromatosis-associated herpesvirus (RFHV), is the most fully characterized of the non-human RV1 rhadinoviruses to date. The complete sequences for a region of the viral genome containing the DNA polymerase and four other adjacent genes have been obtained for two strains of RFHV from different macaque species, *Macaca mulatta* (rhesus) (RFHVMm) and *Macaca nemestrina* (pig-tailed) (RFHVMn) (Rose et al., 2003). The RV2 rhadinovirus lineage consists of a group of more distantly related viruses which co-infect the same Old World primate species (Greensill et al., 2000; Lacoste et al., 2000b; Schultz et al., 2000; Lacoste et al., 2001; Whitby et al., 2003). Of this group, the macaque RV2 rhadinoviruses are the most completely characterized. The complete sequence of the genomes of two strains of the rhesus macaque RV2 rhadinovirus (RRV) have been determined (Alexander et al., 2000; Searles et al., 1999), and the glycoprotein B sequence and a partial sequence of the DNA polymerase gene are available from the virus found in pig-tailed macaques (MneRV2/PMRV) (Auerbach et al., 2000; Schultz et al., 2000). The presence of RV1 and RV2 rhadinoviruses in both Old World monkeys and apes suggests that the two viral lineages have evolved from an ancient non-speciative divergence within an ancestral primate host.

We first identified the macaque RV1 rhadinovirus, RFHV, in retroperitoneal fibromatosis and related subcutaneous fibromatosis lesions of macaques, herein referred to as RF (Rose et al., 1997). RF is a fibroproliferative neoplasm associated with simian AIDS (SAIDS) which has strong similarities to AIDS-KS (Tsai et al., 1990). Like KS, RF lesions are multifocal with increased vascularity, and contain tumor cells exhibiting a characteristic spindle-shaped cell morphology (London et al., 1983; Tsai et al., 1985). We previously detected the presence of both macaque RV1 and RV2 rhadinoviruses in RF tumor lesions by PCR (Schultz et al., 2000; Bielefeldt-Ohmann et al., 2005). In order to study the biology of these viruses and determine their potential roles in the development of RF in macaques, we cloned and sequenced the ORF73 LANA homologs of the RV1 and RV2 rhadinoviruses of the pig-tailed macaque in which the majority of RF cases within the Washington National Primate Research Center (WaNPRC) occurred. We show here that RFHVMn LANA has strong sequence homology to KSHV LANA and exhibits the same structural domains and conserved motifs. We prepared recombinant RFHVMn, MneRV2 and RRV LANA proteins and identified monoclonal antibodies which specifically react with RFHVMn LANA. Using these antibodies, we determined that the spindle-shaped tumor cells within RF lesions are infected with RFHV and express RFHV LANA as a prominent nuclear antigen suggesting that it plays a role in the development and/or maintenance of RF tumors.

Results

Cloning and characterization of ORF 73 LANA of the macaque RV1 rhadinovirus, RFHVMn

A lambda library was prepared from high molecular weight DNA obtained from an archived RF tumor sample of a pig-tailed macaque from the WaNPRC which contained ~1 viral copy/cell of the macaque rhadinovirus, RFHVMn. A lambda clone was identified which contained 10kb of the RFHVMn genome, spanning the *orf73* gene. Sequence analysis revealed a large open reading frame of 3213 bp encoding a 1071-amino-acid protein which showed the highest similarity with the KSHV LANA in a BLAST search. This open reading frame was flanked by sequences upstream and downstream which showed close similarities to ORF72, K13 and K14 of KSHV indicating that the region of the RFHVMn genome containing the LANA locus was identical in structure and gene content to KSHV. Sequence alignment of the LANA homologs of the RFHVMn and KSHV RV1 rhadinoviruses revealed a close similarity in length and structure. Like KSHV LANA, RFHVMn LANA contained an N-terminal region of ~300 aa and a C-terminal region of ~225 aa which were separated by an acidic repeat region of