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Kaposi's Sarcoma-Associated Herpesvirus Contains G Protein-Coupled Receptor and Cyclin D Homologs Which Are Expressed in Kaposi's Sarcoma and Malignant Lymphoma

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A new human herpesvirus was recently identified in all forms of Kaposi's sarcoma (Kaposi's sarcoma-associated herpesvirus [KSHV] or human herpesvirus 8), as well as in primary effusion (body cavity-based) lymphomas (PELs). A 12.3-kb-long KSHV clone was obtained from a PEL genomic library. Sequencing of this clone revealed extensive homology and colinearity with the right end of the herpesvirus saimiri (HVS) genome and more limited homology to the left end of the Epstein-Barr virus genome. Four open reading frames (ORFs) were sequenced and characterized; these are homologous to the following viral and/or cellular genes: (i) Epstein-Barr virus membrane antigen p140 and HVS p160, (ii) HVS and cellular type D cyclins, (iii) HVS and cellular G protein-coupled receptors, and (iv) HVS. Since there is considerable evidence that cyclin D1 and some G protein-coupled receptors contribute to the development of specific cancers, the presence of KSHV homologs of these genes provides support for a role for KSHV in malignant transformation. All ORFs identified are transcribed in PELs and Kaposi's sarcoma tissues, further suggesting an active role for KSHV in these diseases.

Two novel DNA fragments belonging to a previously unidentified human herpesvirus were recently identified in a Kaposi's sarcoma (KS) lesion (17). Extensive sequencing, transmission, and serologic studies demonstrate that these sequences belong to a new human herpesvirus, Kaposi's sarcoma-associated herpesvirus (KSHV), also called human herpesvirus 8 (47). While this virus is generally absent from normal control tissues, it is consistently present in AIDS- and non-AIDS-related KS (13, 17, 19, 24, 46, 60), AIDS- and non-AIDS-related primary effusion (body cavity-based) lymphomas (PELs) (15, 39), and a significant proportion of cases of multicentric Castleman's disease (23, 30, 63). These sequences are also frequently present in normal-appearing tissue adjacent to KS lesions and in lymph nodes and peripheral blood B cells in patients with KS or at high risk for developing KS (5, 17, 48, 52, 62, 69).

Detection of KSHV in lymph nodes, peripheral blood B cells, and a subset of B-cell lymphomas suggests that it is a lymphotropic herpesvirus. The initial sequence analysis data showing partial homology to herpesvirus saimiri (HVS) and Epstein-Barr virus (EBV) are consistent with this hypothesis (17). Both viruses are members of the *Gammaherpesvirinae* subfamily of herpesviruses, which characteristically replicate in lymphoblastoid cells. HVS, a squirrel monkey (*Saimiri sciureus*) virus, can be isolated from the peripheral blood mononuclear cells of healthy animals but causes fulminant T-cell lymphomas in New World primates other than its natural hosts (28). HVS can also immortalize human T cells so that they grow continuously in vitro (10). EBV is a human herpesvirus well known to immortalize B cells in vitro and is associated with malignant lymphomas, including endemic Burkitt's lymphoma, AIDS-related lymphomas, posttransplantation lymphoproliferative disorders, and Hodgkin's disease (44). Since both viruses can lead to the development of malignant lymphomas, it is quite possible that KSHV is a transforming virus which is involved in the development of PELs.

Since the original identification of two small DNA fragments from an AIDS-KS lesion by representational difference analysis, considerable progress has been made in determining the nature of this virus. Cell lines which allow the in vitro culture of the virus and detailed virologic characterization studies have been established (7, 16, 56). A 20.7-kb clone from a KS library has been sequenced and characterized, confirming that KSHV is a gamma-2 herpesvirus, the first member of the genus *Rhadinovirus* known to infect humans (47). In vitro transmission and visualization at the electron microscopic level have also been achieved, providing additional evidence for the viral nature of the KSHV sequences (43, 47, 56, 58). We have now confirmed and extended these studies by sequencing a 12.3-kb fragment (SGL-1) of the KSHV genome obtained from a PEL genomic library and a 12.8-kb fragment (L54) from a BC-1 genomic library with the KS631Bam fragment as a probe. Results from this analysis further support the notion that KSHV is a human herpesvirus most homologous to HVS and that it may have a role in malignant transformation.

Genomic library and cloning. Genomic DNA was obtained from a pathologic specimen of a PEL, corresponding to case 1 previously described (15). The DNA was digested to completion with *Bgl*II restriction endonuclease (Boehringer Mannheim, Indianapolis, Ind.), and the DNA fragments between 9 and 23 kb in length were isolated by agarose gel electrophoresis fractionation. These fragments were cloned into the LambdaGEM-11 vector as per the manufacturer's instructions (Promega, Madison, Wis.). The SGL-1 clone was identified by hybridization to the KS631Bam probe (17) and subsequently

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purified by standard plating methods (40). A second library was constructed with genomic DNA from the BC-1 cell line, which was derived from the PEL from which the SGL-1 clone was obtained (16). After partial digestion with *Sau*3A restriction endonuclease, the 9- to 23-kb partial digestion products were isolated by agarose gel electrophoresis and cloned into the Lambda Fix II vector according to the manufacturer's instructions (Stratagene, Inc., La Jolla, Calif.). This library was also screened by hybridization to the KS631Bam probe. The resulting phage, L54, was independently shotgun cloned and sequenced (12, 41) to confirm the results obtained from SGL-1.

Genomic sequencing. The SGL-1 bacteriophage clone was digested with *Bam*HI, and the eight fragments obtained were isolated by gel electrophoresis and subcloned into the pGem3Z vector (Promega). Similarly, this clone was digested with *Sac*I, and the two larger fragments were subcloned in pGem3Z (see Fig. 1 for *Bam*HI and *Sac*I restriction maps). Sequencing was performed with the *Taq* DyeDeoxy terminator cycle sequencing system with an ABI 373A automated DNA sequencer (Applied Biosystems, Inc., Foster City, Calif.). Both strands were sequenced by primer walking and nested deletions. The regions containing open reading frames (ORFs) 75, 74, 73, and 72 were completely sequenced with an average fivefold redundancy. For sequencing of the L54 DNA, 25-μg aliquots were sheared with 3- to 5-s pulses with a Micro Ultrasonic Cell Disrupter (Kontes) at its minimum setting. After end repair with T4 DNA polymerase and the Klenow fragment of DNA polymerase I (New England Biolabs, Beverly, Mass.), the 1- to 4-kb fraction was purified by agarose electrophoresis. The inserts (100 to 200 ng) were ligated to 40 to 80 ng of M13 replicative form DNA purified by double cesium chloride banding, linearized with *Hin*cII, and treated with calf intestinal alkaline phosphatase. The ligation mixture was electroporated into XL1-Blue cells (E. coli pulser; Bio-Rad Laboratories, Inc., Hercules, Calif.) which were supplemented with fresh tetracycline-induced saturated XL1-Blue cells and plated in top agarose. After an overnight incubation, plaque lifts were screened by hybridization with radiolabeled L54 insert DNA. Positive plaques were mixed with 2 ml of log-phase tetracycline-induced XL1-Blue cells and incubated for 8 h in a 37°C shaker. Single-stranded M13 phage DNA was purified with QIAprep 96 M13 kits (Qiagen, Chatsworth, Calif.). Enough M13 clones were picked to provide eightfold coverage of the phage. Sequencing reactions were performed with -21 M13 dye primer cycle kits (Perkin-Elmer, Foster City, Calif.) according to the manufacturer's instructions. Reaction products were analyzed on ABI 377 sequencers, and the sequence was assembled with the Autoassembler package (Applied Biosystems, Inc.).

Nucleotide composition of the SGL-1 clone. The sequence of this portion of the KSHV genome has an overall G+C content of 54% and an A+T content of 46%. This is similar to the overall G+C content of EBV (60%) and equine herpesvirus 2 (57%), another gamma-2 herpesvirus (66). In contrast, HVS has a high G+C content only in its terminal repeats (H-DNA), and a low G+C content in its coding regions (L-DNA, 35% G+C) (34). The observed/expected CpG dinucleotide ratio is 0.57. A low overall percentage of CpG dinucleotides is a feature common to gammaherpesviruses and is thought to result from 5-methylcytosine deamination of methylated CpG residues. This process may be related to the ability of gammaherpesviruses to maintain a latent state in actively dividing cells (34). The low CpG content identified in this region of KSHV is in contrast to the 0.92 observed/expected CpG ratio for the region extending from KSHV ORF26 through ORF35 (47). This discrepancy is probably due to regional variation in CpG

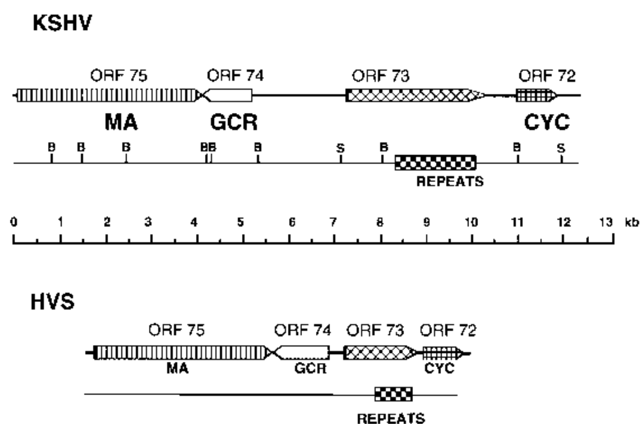

FIG. 1. General structure of the KSHV SGL-1 genomic clone and alignment with the homologous HVS fragment. A map of the fragment sequenced showing the position and orientation of the four ORFs identified is shown in the upper section, and the homologous region of HVS is shown in the lower section of this figure. MA, membrane antigen; CYC, cyclin homolog. The restriction map for *Bam*HI (B) and for *Sac*I (S) is shown, and the region containing multiple internal repeats is depicted by the checkered box.

methylation, since some privileged sites in EBV and HVS seem to be protected from methylation (34). The ORF20 to -35 region has been resequenced from a PEL cell line library (12a) and shows minimal variation from a clone derived from a KS genomic library. Thus, it is unlikely that strain variation accounts for variation in CpG content between these two regions.

Homology, ORF, and translation analysis. Analysis of the DNA sequences for the presence of ORFs and for their translation products was performed with the MacVector 4.1.4 program (Eastman Kodak-IBI, New Haven, Conn.). The BLASTX program was used to search the KSHV DNA sequences for homologous protein sequences (4). Protein sequence databases searched with this program include National Biomedical Research Foundation PIR, SWISS-PROT, GenPept (translated coding sequences from GenBank), and PDB (Brookhaven Protein Data Bank). The sequences were aligned to homologous genes with the ALIGN program from the Ecole pour les Etudes et la Recherche en Informatique et Electronique, Nîmes, France.

Four long ORFs were identified in the SGL-1 clone and designated ORF75, -74, -73, and -72 according to their location and homology to the HVS genome (Fig. 1) (2), consistent with the orientation and nomenclature adopted for KSHV by Moore et al. (47). A map showing the relative locations of these four ORFs is shown in Fig. 1; analyses to determine if additional coding regions present are ongoing.

All four ORFs are colinear and homologous with similar genes in HVS and are present in the same transcriptional orientation. Only one of these genes, ORF75, shows homology to EBV. ORF73 contains unique and repeated sequences similar to ORF73 of HVS. ORF75, -74, and -72 have other viral and/or cellular counterparts as illustrated in Table 1 and as follows.

ORF75. This ORF is located between nucleotides 83 and 3970 of the SGL-1 clone, encoding a putative protein of 1,296 amino acids. The translated product of this sequence shows significant homology to ORF75 of HVS, a 152/160K membrane antigen (14), as well as the corresponding gene products in equine herpesvirus 2 (66) and the alcelaphine herpesvirus 1 (25). It also shows homology to the EBV BNRF1 ORF, encoding the membrane antigen p140 (9). These are thought to

TABLE 1. Homology of ORFs identified in clones SGL-1 and L54 to corresponding viral and cellular genes[a]

KSHV	Gene homolog	% Identity	% Similarity
ORF75	EHV-2-ORF75	34	71
	HVS-ORF75	34	72
	AHV-1-P140	20	65
	EBV-P140	29	67
	D. melanogaster-FGARAT	20	66
	C. elegans-FGARAT	18	62
ORF74-GCR	HVS-ORF74	32	71
	IL-8-R-B-HU	27	74
	IL-8-R-A-HU	25	70
	BLR1	23	66
	EHV-2-U20824	21	66
	LESTR (fusin)	20	66
	HCMV-US28	20	65
ORF72-CYC	HVS-ORF72	33	74
	Cyclin D2	27	64
	Cyclin D3	26	67
	Cyclin D1	24	61
	Cyclin A	14	43

[a] Abbreviations: EHV-2, equine herpesvirus 2; AHV-1, alcelaphine herpesvirus 1; FGARAT, phosphoribosylformylglycinamidine synthase; IL-8-R, IL-8 receptor; HCMV, human cytomegalovirus; BLR1, Burkitt's lymphoma receptor 1; LESTR, leukocyte-derived seven-transmembrane domain receptor. CYC, cyclin.

be nonglycosylated, or poorly glycosylated, structural components of the tegument layer surrounding the capsid. However, the translated product of ORF75 also has full-length homology to the purine biosynthetic enzyme phosphoribosylformylglycinamidine synthase (or formylglycineamide ribotide amidotransferase) from *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Escherichia coli* (59, 67, 70), suggesting that the protein encoded by this ORF may also have a biosynthetic function.

ORF74. This ORF is located between nucleotides 4129 and 5154 of the SGL-1 clone and encodes a putative protein of 342 amino acids. It is transcribed in the opposite direction with respect to the other ORFs in this clone. The putative translation product of this ORF shows homology to the ORF74 of HVS which encodes a G protein-coupled receptor (GCR) homolog (ECRF3) (51). It also shows homology to multiple mammalian GCRs, of which the highest is to the interleukin-8 (IL-8) receptors, but also includes the GCR involved in human immunodeficiency virus cell fusion and entry, and to a lesser degree the type I angiotensin II receptor and the bradykinin receptor (Table 1) (26, 27, 32, 36, 50, 53). There is no counterpart of this gene in the EBV genome, although EBV induces the expression of cellular GCRs (11, 22), which are also homologous to the putative product of KSHV ORF74. As expected for a GCR, the translated product of ORF74 contains seven hydrophobic regions, theoretically corresponding to transmembrane domains, as predicted by the TMpred program from the Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland (33). The KSHV GCR homolog also shares other features with members of this class of receptors, including glycosylation sites in the most N-terminal extracellular fragment and two cysteine residues, in the putative second and third extracellular loops, which are conserved among all GCRs (65).

ORF72. This ORF is located between 569 and 1,343 bp upstream from the 3′ end of the SGL-1 clone and encodes a putative protein of 257 amino acids. This ORF shows homology to ORF72 of HVS which encodes a cyclin D homolog (ECLF2) (51). It also shows homology to multiple mammalian cyclin D proteins, as well as more limited homology to other cyclins (Table 1). Within this ORF, nucleotides 142 to 603 bracket a region with homology to the cyclin box motif (18).

Expression analysis of KSHV in KS and PEL. Expression of the four ORFs identified was evaluated by reverse transcription-PCR (RT-PCR) using RNA obtained from two tissues with KS and the two PEL cell lines (BC-1 and BC-2) previously described (16). Total RNA was isolated by the TRI REAGENT nucleic acid extraction method (Molecular Research Center, Inc., Cincinnati, Ohio) according to the manufacturer's instructions. In order to eliminate any contaminating genomic DNA, the RNA samples were first treated with 2 U of RNase-free DNase I (Boehringer Mannheim) according to the manufacturer's instructions, with subsequent heat inactivation of the enzyme. The RT reaction was carried out on 1 μg of RNA with 0.5 ng of random hexamers and the SUPERSCRIPT reverse transcriptase system (GIBCO-BRL, Gaithersburg, Md.) according to the manufacturer's instructions.

The sequences of the primers and the probes used to detect transcripts from the four long ORFs identified are as follows: ORF75, P2 (5′ primer; 5′-AGGAGCGAGAGAGACGGGAT-3′), P7 (3′ primer; 5′-CCAGGTGCCTGCCCACTTCC-3′), and ORF75 probe (5′-CCTAGCTCTTGCAGCAGAAC-3′); ORF74, P8 (5′ primer; 5′-CGGGGTGCCTTACACGTGG-3′), P9 (3′ primer; 5′-CAGTCTGCAGTCATGTTTCC-3′), and ORF74 probe (5′-TGTGTGCGTCAGTCTAGTGAG-3′); ORF73, P47 (5′ primer; 5′-GCAGTCTCCAGAGTCTTCTC-3′), P16 (3′ primer; 5′-CGGAGCTAAAGAGTCTGGTG-3′), and ORF73 probe 5′-TGGAGGTGTAGTCTGCTGCG-3′); ORF72, P51 (5′ primer; 5′-CACCCTGAAACTCCAGGC-3′), P32 (3′ primer; 5′-GATCCGATCCTCACATAGCG-3′), and ORF72 probe (5′-CGCCACTCTATATGCAAACTG-3′). A primer set specific for the human β-actin cDNA (Stratagene) was used as a quantitative control. The sequence of the β-actin internal oligonucleotide probe is 5′-GGATGTCCACGTCACACTTC-3′. The predicted size of the amplification product resulting from each of these primer sets is depicted in Fig. 2.

The first-strand cDNA samples were subjected to direct PCR with 10 pmol of each 5′ and 3′ primer, in the presence of 1.5 mM $MgCl_2$, and 200 μM deoxynucleoside triphosphates (dNTPs) (KSHV reactions) or 100 μM dNTPs (β-actin reactions). Reactions were performed in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.), and reaction mixtures were subjected to an initial 1.5-min denaturation at 94°C, followed by 30 cycles of denaturation (1 min at 94°C), annealing (2 min at 55°C), and extension (3 min at 72°C). The same reactions were also performed with RNA in the absence of the RT reaction as a control to exclude the presence of genomic DNA as the source of template for the amplified products. Following agarose gel electrophoresis, amplified products were transferred to a nitrocellulose membrane according to the method of Southern (64). Filters were hybridized with a ^{32}P-end-labeled internal oligonucleotide probe as previously described (29) and washed for 15 min at room temperature and then for 10 min at 59°C (ORF73), 57°C (ORF74 and β-actin), 55°C (ORF72), or 54°C (ORF75). The filters were exposed to film at −80°C with an intensifying screen for 45 min to 2 h and for 48 h for the experiments without an RT step.

As shown in Fig. 2, transcripts from all four ORFs were identified in KS and the two PEL cell lines, BC-1 and BC-2. The specificity of this amplification was confirmed by hybridization to a radiolabeled internal oligonucleotide. These bands are a result of RNA amplification and not contamination by genomic DNA, since we failed to identify PCR products when using the DNase-treated RNA preparations in the absence of

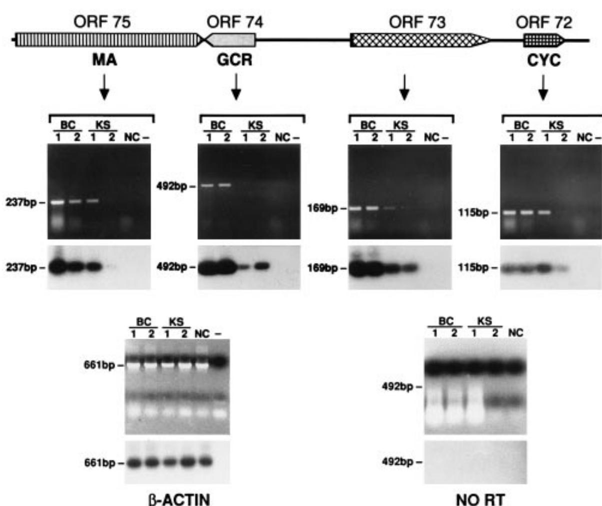

FIG. 2. Expression of KSHV ORFs present in the SGL-1 fragment in KS and PEL. Expression of all four KSHV ORFs was examined by RT-PCR in RNA obtained from two PEL cell lines (BC-1 and BC-2) and from two separate KS specimens (KS-1 and KS-2). The corresponding ORF is indicated by the genomic map in the upper panel. No expression was identified in a KSHV-negative cell line (HL 60) used as a control (NC). The RT-PCR was also performed in the absence of DNA (−). Expression of these ORFs was variable but identifiable by a band in the ethidium bromide-stained gel, as well as by hybridization with an internal radiolabeled oligonucleotide probe of the expected size (indicated by the dashes). The lower left panel shows the RT-PCR for the β-actin gene used as a quantitative control, indicating that all lanes, including the negative control, contain comparable amounts of amplifiable cDNA. The lower right panel shows a PCR with primers for ORF74, with RNA in the absence of the RT step. While a smear is seen in some of the samples, no bands were identified in the ethidium bromide-stained gel at the expected position, or after hybridization with an internal oligonucleotide probe, excluding the presence of viral DNA as a source of template for PCR. This result was confirmed with primers for ORF75, -73, and -72 (not shown).

the RT reaction, even after hybridization with an internal oligonucleotide probe and longer autoradiographic exposures (Fig. 2, no-RT panel). For the most part, the KSHV transcripts appear to be more abundant in the PELs than in KS, which is consistent with the higher genomic copy number in the former (15), although this is a rough estimate, since quantitative PCR was not performed. All specimens, including the KSHV-negative control, have comparable amounts of RNA as seen with the β-actin set of primers and probe. Expression of all four ORFs was confirmed in the BC-1 and/or BC-2 cell lines by Northern (RNA) blot hybridization, excluding the possibility of RT-PCR artifacts (not shown).

Implications of the presence and expression of KSHV GCR and cyclin homologs. Four complete ORFs were identified within the KSHV fragment sequenced in this study. Only one of these ORFs is present in the EBV genome, while HVS possesses analogous genes in the same order and orientation to all four KSHV ORFs identified. This suggests that KSHV is more closely related to HVS than to EBV, a finding that is consistent with the formal phylogenetic analysis of conserved amino acid sequences (47). The genomes of EBV and HVS are largely conserved and colinear, with the exception of specific sets of genes, notably including those with important pathophysiologic functions such as lymphoid immortalization and transformation. For example, the EBV LMP and EBNA genes are not found in HVS, and the HVS transforming gene, STP, is not present in the EBV genome. This also may be the case for ORF74 and -72, which encode a GCR and a cyclin homolog, respectively. While neither of these is present in the EBV genome, expression of cellular members of both the GCR and cyclin D families is induced by EBV-encoded proteins (8, 11, 22).

GCRs represent a very large and diverse family of molecules, responding to a variety of hormone and neurotransmitter agonists, ranging from small biogenic amines like epinephrine and histamine to peptides like bradykinin and large glycoprotein hormones such as luteinizing and parathyroid hormones (65). The KSHV GCR homolog has structural features believed to be functionally important for this class of receptors. Many members of this class of receptors are involved in cell growth and differentiation, and specific members of this family have been found to be involved in malignant transformation, including the human *mas* oncogene which encodes an angiotensin receptor and is tumorigenic in nude mice (35, 72), and several others which have the ability to transform fibroblasts in an agonist-dependent manner (3, 31, 37). Furthermore, activating mutations of the thyroid-stimulating hormone receptor have been found in thyroid adenomas and carcinomas (54, 57).

The closest cellular homologs to ORF74, the KSHV GCR, are the IL-8 receptor types A and B, and the closest viral homolog to this protein is the HVS *ECRF3* gene, which has been shown to encode a functional IL-8 receptor (1). IL-8 belongs to the α chemokine family of molecules, which are structurally related 70- to 90-amino-acid polypeptides involved in inflammation. Thus, it is likely that the KSHV GCR may function as a chemokine receptor. A functional characterization of this receptor, which is currently ongoing in our laboratory, is important for understanding the role of KSHV in KS, since IL-8 is a potent angiogenic factor and KS cells have been found to express appreciable levels of IL-8 (61). Furthermore, EBV-immortalized lymphoblastoid cells and some neoplastic B cells have also been found to produce IL-8 (20, 21, 42, 71), although little is known regarding the presence of IL-8 receptors on B cells and their response to IL-8. Interestingly, the KSHV GCR is also homologous to another member of this family of receptors, the fusin protein, a necessary cofactor for human immunodeficiency virus fusion and cell entry which has been recently described (27). This receptor had been previously identified by several investigators, but its natural ligand remains unknown (26, 32, 36, 53). This finding raises the possibility that the KSHV GCR may also be involved in some viral-cellular interactions.

The putative protein encoded by ORF72 is homologous to the HVS cyclin homolog and to multiple mammalian cyclins, in particular to members of the cyclin D family. Cyclins are required for cellular division and thus play a key role in cellular proliferation (55). Furthermore, one of the human cyclins, cyclin D1, is the PRAD1 oncogene implicated in the development of certain parathyroid tumors (6, 49) and hepatocellular carcinomas (73). Cyclin D1 is also the gene involved in the bcl-1 translocation breakpoint present in mantle cell lymphomas (68). The HVS cyclin has been found to be functional, as it associates with cdk6 and is able to activate protein kinase activity (38). In vitro functional studies show that KSHV cyclin has kinase activity as demonstrated by the phosphorylation of the retinoblastoma protein leading to its inactivation (18).

All four ORFs identified were expressed at the RNA level in the KS and PEL specimens analyzed. The PEL cell cultures studied (BC-1 and BC-2) are composed mainly of latently infected proliferating cells but have a small proportion of cells which are permissive for virus replication, as documented by the appearance of cytopathic changes in these cells, the ability to transmit the virus, and the presence of viral particles containing KSHV DNA in the culture supernatants (16, 43, 47).

Thus, perhaps unlike the BCBL-1 cells reported by Renne et al. (56), the BC-1 and BC-2 cells do not appear to be tightly latent, and expression of ORF72 to -75 in these cell lines could be a result of either latent or lytic infection. However, induction experiments using 12-O-tetradecanoylphorbol-13-acetate (TPA) and phosphonoacetic acid demonstrate that at least the cyclin gene is expressed during latent infection in the BC-1 cell line (27a). Our finding of expression of these four ORFs in KS appears to contrast with the recent study by Zhong et al. (74), in which expression of only two transcripts was identified by Northern blot analysis using probes spanning 120 kb of the KSHV genome and apparently including the region reported in this manuscript. While neither of these transcripts corresponds to the ORFs described here, this discrepancy is easily explained by the large difference in sensitivity of the Northern blot analysis performed by Zhong et al. and our RT-PCR analysis. Furthermore, the amount of KSHV DNA is highly variable from one KS lesion to another (17), and thus the amount of KSHV RNA is likely to be likewise variable. Thus, differences in the KS samples analyzed may account for detection of specific transcripts in some but not in other KS specimens. As part of a general effort to understand the patterns of KSHV gene expression (45, 56), experiments are now ongoing to determine whether expression of the four ORFs reported here is associated with latent or lytic infection in PELs and KS.

The presence and expression of KSHV GCR and cyclin homologs, both of which are genes that control cellular proliferation and/or differentiation, provide strong circumstantial evidence that KSHV is an oncogenic virus. This finding supports the epidemiologic evidence that KSHV plays an active role in the pathogenesis of Kaposi's sarcoma and PELs.

Nucleotide sequence accession numbers. The sequences of the ORFs having homology to known genes have been submitted to GenBank under the following accession numbers: U24269 (ORF75; membrane antigen homolog), U24275 (ORF74; GCR homolog), U24276 (ORF72; cyclin homolog), and U52064 (ORF73).

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