Kaposi’s Sarcoma-associated Herpesvirus-encoded v-Cyclin Triggers Apoptosis in Cells with High Levels of Cyclin-dependent Kinase 6

Päivi M. Ojala,1 Marianne Tiainen, Petri Salven, Tanja Veikkola, Esmeralda Castaños-Vélez, Ronit Sarid,1 Peter Biberfeld, and Tomi P. Mäkelä

Cell Cycle Laboratory [P. M. O., M. T., T. P. M.] and Molecular/Cancer Biology Laboratory [T. V.], Haartman Institute, 00014 University of Helsinki, Helsinki, Finland; Department of Oncology, University of Helsinki, and Helsinki University Central Hospital, 00029 HUKS, Helsinki, Finland [P. S.]; Immunopathology Laboratory, Institute for Pathology and Oncology, Karolinska Institute/Hospital, S-171 76 Stockholm, Sweden [E. C.-V., P. B.]; and Department of Pathology and Division of Epidemiology, Columbia University College of Physicians and Surgeons, New York, New York 10032 [R. S.]

ABSTRACT

Kaposi’s sarcoma-associated herpesvirus (KSHV) has a key etiological role in development of Kaposi’s sarcoma (KS). v-Cyclin is a KSHV-encoded homologue to D-type cyclins that associates with cellular cyclin-dependent kinase 6 (CDK6). v-Cyclin promotes S-phase entry of quiescent cells and has been suggested to execute functions of both D- and E-type cyclins. In this study, expression of v-cyclin in cells with elevated levels of CDK6 led to apoptotic cell death after the cells entered S phase. The cell death required the kinase activity of CDK6 because cells expressing a kinase-deficient form of CDK6 did not undergo apoptosis upon v-cyclin expression. Studies on the mechanisms involved in this caspase-3-mediated apoptosis indicated that it was independent of cellular pRb or p107. In contrast, the KSHV-encoded v-Bcl-2 efficiently suppressed v-cyclin/CDK6-induced apoptosis, demonstrating a marked difference in the antiapoptotic properties of c-Bcl-2 and v-Bcl-2. In KS lesions, high CDK6 expression was confined to a subset of cells, some of which displayed signs of apoptosis. These results suggest that v-cyclin may exert both growth-promoting and apoptotic functions in KS, depending on factors regulating CDK6 and v-Bcl-2 levels.

INTRODUCTION

Several oncogenic viruses encode both growth-promoting and antiapoptotic proteins with a potential role in tumor induction (reviewed in Ref. 1). The KSHV/human herpesvirus 8 (HHV8) (2) is consistently found in spindle cells of all forms of KS lesions, suggesting a key etiological role in the development of KS (reviewed in Ref. 3). The majority of the KS spindle cells are latently infected with KSHV (4–6), but lytic infection is detected only in a small fraction (0.5–1%) of cells (6, 7). The KSHV genome contains homologues to several cellular genes, including bcl-2, FLIP, and cyclin D, which may modulate apoptosis and cell proliferation (reviewed in Ref. 8).

The KSHV-encoded v-cyclin (9), also termed ORF72, vCYC, KSHV-cyclin, v-cyclin D, and K-cyclin (8), is expressed both during latency and the lytic viral replication cycle (10, 11). It is closely related to cellular D-type cyclins, which regulate progression through the G1 phase of the cell cycle in complex with CDK4 or CDK6 (reviewed in Ref. 12). The primary function of cyclin D complexes is apparently inactivation of the retinoblastoma protein (pRb). In malignancies, cell cycle progression is often deregulated by mutations in these pRb G1 checkpoint pathway genes.

v-Cyclin associates specifically with CDK6 to form a functional kinase complex when it is transfected in COS or U2OS cells (13–16) and also in insect cell lysates (13–17). Intriguingly, the in vitro substrate specificity of the v-cyclin/CDK6 complex is extended from cellular cyclin D/CDK6 complexes and includes not only pRb (glutathione S-transferase-pRb) but also histone H1, cdc25a, Id-2, and p27 (13–17). Ectopic expression of v-cyclin induces several growth-promoting effects, including pRb phosphorylation (9); evasion of a G1 arrest by the cellular CDK inhibitors p16, p21, and p27 (17); and induction of S phase entry in quiescent NIH 3T3 cells (17). The evasion of p27 arrest is mediated by phosphorylation of p27 by the v-cyclin/CDK6 complex, which triggers p27 degradation (15, 16).

v-Cyclin can apparently overcome normal cell cycle control mechanisms by executing functions of both D and E cyclin complexes and, therefore, could be implicated in oncogenic properties of KSHV (18). Because v-cyclin apparently requires cellular CDK6 to exert these functions, here we investigated the role of CDK6 in v-cyclin induced deregulation of cell cycle progression. Expression of v-cyclin in cells with elevated levels of CDK6 accelerated entry into S phase but also led to apoptotic cell death, suggesting that v-cyclin may exert both growth-promoting and apoptotic functions in KS.

MATERIALS AND METHODS

Expression Vectors. The previously described expression vectors used in this study were: Rc-cyclD1-HA (19), pCMV-CDK6-HA and pCMV-CDK4-HA (20), pSG5-v-Bcl-2-HA (Ref. 21; kindly provided by Dr. J. M. Hardwick Johns Hopkins University, Baltimore, Maryland, MD), pCMV-HA-E2F-1 (22), and pCMV-HA-FLIP (23).

Nontagged versions of CDK6, CDK6DN, and v-Bcl-2 were expressed from pCMV-CDK6, pCMV-CDK6DN, and pcDNA3-Bcl-2 (Ref. 24; kindly provided by Dr. L. C. Andersson Haartman Institute, University of Helsinki, Finland) respectively.

The cDNA of a myc-tagged viral cyclin (kindly provided by Drs. R. Sarid and P. Moore, Columbia University, NY, NY), was subcloned into pC-neo (Promega, Madison, WI). The viral FLIP cDNA (kindly provided by Dr. Chris Bosshoff, Institute Cancer Research, London, United Kingdom) sequence contained a T→C difference to the published sequence at position 20 (GenBank accession no. U90534; Ref. 25). Following PCR mutagenesis to change this position to a T, the cDNA was subcloned into pC-neo with an NH2-terminal HA tag (pAHc-v-FLIP).

Antibodies and Reagents. Mouse monoclonal antibodies recognizing the myc epitope (9E10) or HA epitope (12CA5) were from Babco Inc. (Berkeley, CA), α-CDK6 rabbit polyclonal (C-21) was from Santa Cruz Biotechnology Inc. (Santa Cruz, CA), α-β-galactosidase rabbit polyclonal serum was from Chemicon International Inc. (Temecula, CA), and α-Bcl-2 mouse monoclonal antibody was from PharMingen (San Diego, CA). Bisbenzimide Hoechst 33342 was from Sigma Chemical Co. (St. Louis, MO), and staurosporine, Ac-DEVD-pNA, colorimetric CPP32 substrate, and DEVD-CHO CPP32 inhibitor were all products of Biomol Research Laboratories, Inc. (Plymouth Meeting, PA).

Cell Culture and Transfections. Human osteosarcoma cell lines U2OS and SaOS-2, mouse myoblast cells C2D12, human embryonic 293 cells, and

Received 4/8/99; accepted 8/4/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This study was supported by grants from the Academy of Finland, the University of Helsinki, the Finnish Cancer Society, the Finnish Cancer Institute, the Sigrid Juselius Foundation, and the BIOMED-2 Concerted Action on the “Pathogenesis of AIDS Kaposi’s Sarcoma” (Contract BMH4-97-2302).

2 This study was supported by grants from the Academy of Finland, the University of Helsinki, the Finnish Cancer Society, the Finnish Cancer Institute, the Sigrid Juselius Foundation, and the BIOMED-2 Concerted Action on the “Pathogenesis of AIDS Kaposi’s Sarcoma” (Contract BMH4-97-2302).

3 Present address: Faculty of Life Sciences, Bar Ilan University, Ramat-Gan 52900, Israel.

4 The abbreviations used are: KSHV, Kaposi’s sarcoma-associated herpesvirus; KS, Kaposi’s sarcoma; CDK, cyclin-dependent kinase; HA, hemagglutinin; BrdUrd, 5-bromo-2-deoxyuridine; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling.
resolved by SDS-PAGE (12%) and immunoblotted with v-cyclin and CDK6, or cyclinD1 and CDK6. Total lysates of transfected cells were of U2OS cells at 28 h posttransfection. Cells were transfected with v-cyclin alone, contrast micrographs. Arrows (left), cells with altered morphology. Right, phase contrast micrographs. Arrows, cells with altered morphology. C, Western blotting analysis of U2OS cells at 28 h posttransfection. Cells were transfected with v-cyclin alone, v-cyclin and CDK6, or cyclinD1 and CDK6. Total lysates of transfected cells were resolved by SDS-PAGE (12%) and immunoblotted with α-CDK6 antibodies.

COS-7 were maintained in DMEM supplied with 10% (w/v) FCS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine. Transient transfections were performed using calcium phosphate coprecipitation according to standard protocols (26) with a total DNA amount of 40 μg including 2.5 μg of pCMV-β-gal DNA. DNA precipitates were washed at 20 h, and the cells were placed in fresh medium. Cells were analyzed 12–52 h later. Transfection efficiency was monitored by β-galactosidase staining or activity assay by using chlorophenol red-β-D-galactopyranoside (Roche, Basel, Switzerland) as the substrate according to manufacturer and measured by absorbance at 574 nm. Transfection efficiency was between 10 and 30%.

Western Blotting. The cells were lysed into 1% NP40 lysis buffer [20 mM NaPO4 (pH 7.4), 1% NP-40, 250 mM NaCl, 5 mM EDTA, 5 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, and 1.5 μg/ml aprotinin]. Forty μg of total proteins were analyzed by 12% SDS-PAGE and blotted according to standard protocols.

Indirect Immunofluorescence and Immunohistochemistry. Transfected cells on coverslips were fixed with 3.5% (w/v) paraformaldehyde, permeabilized with 0.1% TX-100 for 5 min, and labeled as described elsewhere (27). DNA was stained with Hoechst 33342 (0.5 μg/ml) for 5 min, and the coverslips were mounted in 50% glycerol in PBS on glass slides and viewed under a fluorescence microscope. Tissue sections from two paraffin-embedded and two frozen, acetone-fixed KS lesions were analyzed by immunohistochemistry essentially as described previously (28) using a rabbit polyclonal α-CDK6 antibody (C-21; Santa Cruz Biotechnology).

**BrdUrd Assay.** At desired times after transfection, 30 μM BrdUrd (Sigma) was added in the growth medium and incubated for 2 (see Fig. 1A) or 6 h (see Fig. 1B). The cells were fixed with 99% (w/v) ethanol plus 5% (w/v) acetic acid for 20 min and treated with 2 M HCl for 10 min. Subsequently incorporated BrdUrd was detected with a monoclonal α-BrdUrd (DAKO, Glostrup, Denmark) in double labeling with α-β-Gal (see above). At least 200 transfected (β-galactosidase positive) cells were scored for BrdUrd incorporation.

**Apoptosis Assays.** For the quantitative apoptosis assay, percentages of nuclei that displayed apoptotic morphology in Hoechst-staining were determined visually, and the maximal apoptosis (obtained in the v-cyclin-/CDK6-expressing cells) was given a value of 100 (see Fig. 3C); other values are relative to this. At least 500 nuclei were scored in a double-blind count for apoptotic morphology. In the case in which apoptosis of transfected cells was determined (see Fig. 4), at least 200 transfected cells were scored. For the CPP32 assay, 52 h after the removal of the DNA precipitates, the detached and adherent cells were pooled and used for the CPP32 assays essentially as described previously (29) using 60 μM of the CPP32 inhibitor DEVD-CHO in indicated experiments. TUNEL was performed on frozen, parafomaldehyde-fixed KS sections to detect apoptotic cells using an **in situ** cell death detection kit according to the manufacturer’s instructions (Roche).

**RESULTS**

**Coexpression of CDK6 Enhances v-Cyclin-induced S-Phase Entry.** To analyze whether high levels of CDK6 would affect v-cyclin induced cell cycle promotion, we analyzed S-phase entry of U2OS cells transfected with the v-cyclin alone or v-cyclin with CDK6 by BrdUrd incorporation (Fig. 1A). In accordance with previous studies, v-cyclin expression alone promoted S-phase entry of cycling U2OS cells (17), presumably by shortening $G_1$. v-cyclin was found to be more efficient than cyclin D1 in this function (Fig. 1A). Because v-cyclin associates specifically with CDK6, the increase in S-phase cells was presumed to result from v-cyclin associating with limiting amounts of endogenous CDK6 in U2OS cells (Fig. 1C; Ref. 30). Accordingly, ectopic CDK6 expression significantly increased the number of BrdUrd-positive cells when transfected together with either v-cyclin or cyclin D1, but the effect of CDK6 on v-cyclin was slightly more efficient (Fig. 1A). These results indicate that both v-cyclin and cyclin D1 are capable of promoting S-phase entry when complexed with CDK6.

**Fig. 1.** Induction of S phase by the v-cyclin/CDK6 complex. A, fractions of BrdUrd-positive U2OS cells transfected with the expression vectors indicated on the right analyzed at 14 h posttransfection. The BrdUrd pulse was for the last 2 h prior to fixation. Columns, means of triplicate samples; bars, SD. B, U2OS cells transfected with v-cyclin (v-cyc) or v-cyclin and CDK6 (v-cyc + CDK6) were analyzed at 28 h posttransfection by immunofluorescence with anti-BrdUrd staining (left). The BrdUrd pulse was for 6 h at 10–16 h posttransfection. DNA was stained with Hoechst 33342 (middle). Right, phase contrast micrographs. Arrows, cells with altered morphology. C, Western blotting analysis of U2OS cells at 28 h posttransfection. Cells were transfected with v-cyclin alone, v-cyclin and CDK6, or cyclinD1 and CDK6. Total lysates of transfected cells were resolved by SDS-PAGE (12%) and immunoblotted with α-CDK6 antibodies.

**Fig. 2.** Cells expressing both v-cyclin and CDK6 undergo apoptosis. Caspase-3 (CPP32) activity from U2OS cell lysates transfected with expression vectors indicated on the right was determined based on the ability to cleave Ac-DEVD-pNA. DEVD-CHO peptide at 60 μM was used as a specificity control in the indicated samples. Columns, means of triplicate samples; bars, SD.
Expression of v-Cyclin in Cells with High Levels of CDK6 Triggers Apoptosis. A significant number of BrdUrd-positive cells in the v-cyclin/CDK6 transfection displayed striking morphological alterations consistent with apoptosis (Fig. 1B). Induction of apoptosis was substantiated by an increased caspase-3 (CPP32) activity in v-cyclin-/CDK6-transfected cell lysates (Fig. 2) and TUNEL staining (data not shown). The caspase-3 activity was comparable with staurosporine-induced apoptosis (31, 32) of the U2OS cells (Fig. 2) and was inhibited by the specific peptide inhibitor, DEVD-CHO. Induction of CPP32 protease activity is a key early event in apoptosis (33), implying that the v-cyclin-/CDK6-complex activates a common mammalian cell death pathway. The cell morphology and the activation of CPP32 indicate that ectopic expression of v-cyclin with CDK6 in U2OS cells results in apoptosis after S-phase entry.

v-Cyclin-induced Apoptosis Is Dependent on CDK6 Kinase Activity. Apoptosis of v-cyclin-/CDK6-transfected cells increased with time following transfection. It was first detected 12 h posttransfection concomitantly with detectable v-cyclin expression (data not shown), and at 52 h, the chromatin of virtually all v-cyclin/CDK6 double-positive cells appeared apoptotic with a single bright spherical clump (Fig. 3A, arrowhead) or as several condensed fragments (Fig. 3A, arrow).

v-Cyclin-mediated cell death was clearly dependent on high levels of CDK6 (Fig. 1C) because it was not detected when v-cyclin was transfected alone (Fig. 3B). In contrast, virtually all v-cyclin-positive cells in the v-cyclin/CDK6 transfection were apoptotic, suggesting that even low levels of v-cyclin drive cells with an excess of CDK6 to apoptosis. The apoptosis was specific for the viral cyclin D homologue because it was not detected in cells transfected with cyclin D1/CDK6 (Fig. 3, B and C).

The inability of a kinase-deficient mutant of CDK6 (CDK6DN; Ref. 20) to induce apoptosis in combination with v-cyclin (Fig. 3, B and C) indicates that the kinase activity of CDK6 was required. When CDK4 was cotransfected with v-cyclin, a slight but reproducible increase in the proportion of apoptotic cells was observed (Fig. 3C), suggesting that CDK4 also did form functional complexes with v-cyclin when overexpressed in U2OS cells but markedly less efficiently than CDK6, in accordance with previous studies (13, 14).
v-Cyclin/CDK6-mediated Apoptosis Occurs in High Serum and Is p53 and pRb Independent. Apoptosis resulting from deregulated expression of E1A or Myc only occurs when E1A or Myc is induced in the presence of limiting growth factors (34, 35). On the other hand, expression of E2F-1, especially together with DP-1 (36), induces apoptosis, even in the presence of a full complement of serum growth factors, although less efficiently (37, 38). When cell death induced by v-cyclin/CDK6 was compared with E2F-1/DP-1 apoptosis, cell death by v-cyclin/CDK6 was significantly higher in both U2OS and C2C12 mouse myoblast cells (Fig. 4A). Moreover, apoptosis induced by v-cyclin/CDK6 in U2OS cells was unaffected by serum levels, although a slight decrease in apoptosis was seen in C2C12 cells at high serum concentration (Fig. 4B). To address the role of pRb and p53 in the v-cyclin/CDK6-induced apoptosis, we tested SaOS-2 osteosarcoma cells (with mutant p53 and pRb), COS-7 cells (p53 and pRb inactivated by the SV40 large T-antigen), and C2C12 mouse myoblasts (wild-type p53 and pRb as well as in U2OS cells). We also transfected human embryonic 293 cells and,

![Image](https://example.com/image.jpg)

**Fig. 6.** A subset of Kaposi’s sarcoma cells with high CDK6 expression display morphological signs of apoptosis. A, α-CDK6 immunostaining of a section from a KS lesion demonstrating reactivity in the tumor area but not the surrounding tissue (bottom right of micrograph; original magnification, ×20). B, a section adjacent to the one shown in A was stained as a control by omitting the primary antibody. Original magnification, ×20. C, α-CDK6 immunostaining of a section from a KS lesion demonstrating a low but specific overall expression. Several cells with high levels of CDK6 display signs of apoptosis (arrows), but morphologically unaltered cells can also be found (arrowhead). Original magnification, ×400. D, TUNEL staining showing apoptotic cells in the KS lesion stained in C and E. Arrows, TUNEL-positive cells. Original magnification, ×1000. E, staining as in C from a separate field of the KS lesion at a higher magnification. Arrow, a cell with high CDK6 associated with morphological signs of apoptosis. Original magnification, ×1000.

as in all of the above cell types, the active complex of v-cyclin and CDK6 induced cell death (shown for SaOS-2 in Fig. 4B), whereas v-cyclin/CDK6DN or cyclinD1/CDK6 did not. Thus, apoptosis triggered by v-cyclin and CDK6 occurs in various cell types, independent of either p53 or pRb status.

Antia apoptotic Activity of v-Bcl-2 in v-Cyclin-/CDK6-induced Apoptosis. To test for the ability of v-Bcl-2 and v-FLIP as well as cellular Bcl-2 to protect U2OS cells from the v-cyclin/CDK6-induced apoptosis, we included HA-tagged v-bcl-2, bcl-2, or v-FLIP in transfections with v-cyclin/CDK6. The expression of c-Bcl-2 and v-Bcl-2 was confirmed by Western blotting analysis using α-Bcl-2 and α-HA antibodies, respectively (Fig. 5C). Double labeling with α-HA and α-CDK6 antibodies identified cells coexpressing v-Bcl-2 and CDK6 (Fig. 5A). Parallel experiments demonstrated that >80% of cells expressing CDK6 also contained v-cyclin at levels similar to those shown in Fig. 3A. Most double-positive cells did not display an apoptotic morphology (Fig. 5A, arrowheads) demonstrating that expression of v-Bcl-2 suppressed apoptosis by v-cyclin/CDK6 significantly (quantitated in Fig. 5B), although some apoptotic cells were also seen (Fig. 5A, arrow). Interestingly, transfection of c-bcl-2 or HA-v-FLIP constructs did not inhibit v-cyclin/CDK6 apoptosis.
(Fig. 5B), indicating that v-cyclin/CDK6 apoptosis is specifically suppressed by the KSHV-encoded v-Bcl-2.

**A Subset of KS Cells with High CDK6 Expression Display Morphological Signs of Apoptosis.** The cell death mediated by v-cyclin/CDK6 required high levels of CDK6. To investigate CDK6 expression in KS lesions, sections from four KSHV-positive KS nodules were immunostained for CDK6. A weak but specific overall CDK6 staining was observed in KS lesions (Fig. 6, A and C), but not in the surrounding uninvolved tissue (Fig. 6A). Moreover, a subset of cells in the lesion (~1%) expressed high levels of CDK6 (Fig. 6C), comparable with that seen in cells transfected with CDK6 (data not shown). The high CDK6 signal was most prominent in highly vascularized areas of the lesion containing infiltrating lymphoid cells. Interestingly, a significant proportion of high CDK6 expressing cells displayed morphological signs of apoptosis (Fig. 6, C and F; arrows). TUNEL staining of the same lesion indicated that apoptotic cells (Fig. 6, D and F, arrows) were also mostly confined to the highly vascularized areas containing infiltrating lymphoid cells as reported. These results suggest that high CDK6 expression in a subset of cells in KS lesions is associated with apoptosis.

**DISCUSSION**

These results demonstrate that the efficiency of KSHV v-cyclin to promote S-phase entry is dependent on CDK6 levels. CDK6 also enhanced the ability of cyclin D1 to promote S phase, and indeed, v-cyclin was indistinguishable from cyclin D1 in this respect. In contrast, only cells expressing v-cyclin/CDK6 underwent massive apoptosis, which was never detected in cyclin D1 transfections, demonstrating clearly distinct functions for these cyclins and suggesting that the v-cyclin/CDK6 complex deregulates the cell cycle by taking over functions of both D- and E-type cyclin complexes (17).

The difference between v-cyclin and cyclin D1 in inducing apoptosis in our system is unlikely to be due to different expression levels because v-cyclin was expressed at low levels and was barely detectable in Western blots (data not shown), whereas cyclin D1 was produced at high levels. Another possibility for the inability of cyclin D1/CDK6 complexes to induce apoptosis would be inhibiting levels of CDK inhibitors such as p21 and p27, which would not affect v-cyclin/CDK6 complexes (15–17). This would imply that endogenous levels of these inhibitors would be sufficient to inhibit the transfected cyclin D1/CDK6 complexes. Previously, cyclin D1 has been reported to induce apoptosis in certain cell lines (39) and in terminally differentiated neurons (40). The apparent discrepancy of these reports and our results could be due to cell type specificity and also to the observation that cyclin D1-induced apoptosis generally requires limiting growth factors, unlike v-cyclin/CDK6-induced apoptosis.

Cell death by v-cyclin/CDK6 was, in several ways, similar to apoptosis induced by E2F-1 (37, 41). It occurred after S-phase entry, suggesting that the v-cyclin/CDK6 complex drives the cells into S phase inappropriately, and this could initiate the apoptotic signal. It was also insensitive to genetic backgrounds (p53, pRb), indicating that it is at least partly mediated via a p19ARF-p53 independent pathway similarly to E2F-1 (38, 42, 43). The occurrence of cell death in this model, v-cyclin/CDK6 apoptosis is specifically independent of Bcl-2 and on a different pathway (reviewed in Ref. 44) or that v-cyclin/CDK6 can selectively inactivate cellular Bcl-2. The antiapoptotic activity of Bcl-2 and v-Bcl-2 was indistinguishable in other systems, supporting the latter model. In this model, v-Bcl-2 would be insensitive to this inactivation. The role of apoptosis in KSHV infection was recently demonstrated in vitro in 293 cells using viral isolates from primary KS lesions (45). Interestingly, this apoptosis was also protected by v-Bcl-2 similarly to the v-cyclin/CDK6-induced apoptosis. Apoptosis in KS lesions is limited predominantly to tumor-infiltrating lymphoid cells. Previous studies implicating CDK6 as the partner for v-cyclin (13–17) have not investigated the expression levels of CDK6 in KS lesions. This study in a limited material indicates that CDK6 is detected in KS spindle cells throughout the lesion. Interestingly, some of the predominantly lymphoid cells with high CDK6 expression displayed morphological signs of apoptosis. Using TUNEL staining, we detected apoptosis in KS lesions in similar cells, as have others. Moreover, lymphoid cells in KS lesions express KSHV v-cyclin (46) and lytic markers of KSHV (6, 7). Taken together, these results indicate that v-cyclin may elicit either growth promoting or apoptotic signals in KS, depending on factors in the cellular microenvironment regulating CDK6 and v-Bcl-2 levels.

**ACKNOWLEDGMENTS**

We thank Drs. Patrick Moore and Yuan Chang, Marie Hardwick, Chris Bosshof, Erwin Tschachler, and Päivi Miettinen for reagents; Marja Jäättelä and Kari Alitalo for fruitful discussions and critical reading of this manuscript; Leif Andersson and the Mäkelä laboratory for many helpful suggestions; Birgitta Tjäder for excellent technical assistance; and Antti Huittinen for digital imaging.

**REFERENCES**


---

*6* P. M. Ojala and T. P. Mäkelä, unpublished results.
Kaposi's Sarcoma-associated Herpesvirus-encoded v-Cyclin Triggers Apoptosis in Cells with High Levels of Cyclin-dependent Kinase 6


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/19/4984

Cited articles
This article cites 45 articles, 26 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/19/4984.full#ref-list-1

Citing articles
This article has been cited by 22 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/59/19/4984.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/59/19/4984.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.