Cyclin encoded by KS herpesvirus

Sir—Kaposi’s sarcoma-associated herpesvirus (KSHV or HHV8) has been strongly implicated in the development of Kaposi’s sarcoma and primary effusion lymphomas. This herpesvirus was initially described in Kaposi’s sarcoma-associated with AIDS patients but has since been found in all epidemiological forms of Kaposi’s sarcoma. KSHV is a gammaherpesvirus, similar to other oncogenic herpesviruses such as the New World monkey virus, herpesvirus saimiri, and Epstein–Barr virus. Genomic sequencing of KSHV has thus far failed to identify sequence similarity to genes of Epstein–Barr virus or herpesvirus saimiri which are known to have important oncogenic functions. P S M, unpublished data). In this report, we report here that KSHV contains an open reading frame (ORF 72) with sequence similarity to cellular cyclins, in particular the D-type cyclins (a in the figure). (Herpesvirus saimiri also has an open reading frame with similarity to cyclin.)

Various lines of evidence suggest that deregulated expression of cellular D-type cyclins is linked to the development of tumours in humans. Cyclin D proteins are regulatory subunits which activate cellular kinases (CDKs) to phosphorylate checkpoint molecules such as the retinoblastoma-tumour suppressor protein (RB). The similarity of KSHV ORF 72 to cellular D-type cyclins suggests that its encoded product, KSHV cyclin, could also stimulate such kinases resulting in their unlicensed activation and, consequently, cause deregulation of cellular proliferation inherent to KSHV-associated diseases.

To determine whether KSHV-cyclin could activate kinases and perturb control of cell growth in a manner analogous to aberrant expression of D-type cyclins, we engineered an epitope-tagged (c-myc epitope) version of KSHV ORF 72 driven by the cytomegalovirus immediate early promoter. On transfection into COS-1 cells, this construct gives rise to expression of a polypeptide of relative molecular mass 28,000 that, like the D-type cyclins, is localized to the cell nucleus. The KSHV-cyclin protein is associated with kinase activity capable of phosphorylating RB in vitro at authentic sites, including those previously shown to be a hallmark of RB inactivation (b in the figure). This supports the hypothesis that KSHV-cyclin-associated kinases could mediate the functional inactivation of RB and thereby overcome the cell-cycle arrest imposed by RB.

To test this possibility directly, we co-expressed the KSHV-cyclin together with RB in SAOS-2 osteosarcoma cells which, due to the mutation of both RB alleles, lack expression of endogenous RB. On transfection of wild-type RB, these cells stop growing, resulting in a spreading and senescent-appearing morphology. Co-expression of KSHV-cyclin in these cells abrogates this response, as previously noted with cellular cyclins. Moreover, the RB detected in cell lysates from such transfected cells shows a retarded migration characteristic of hyperphosphorylated, inactive RB (c in the figure). Taken together, our findings imply that the ectopic expression of KSHV-cyclin in latently infected host cells can inappropriately activate kinases whose activity normally requires cellular cyclins. This in turn can allow such cells to overcome growth-restriction checkpoints, such as that imposed by RB. Although direct evidence for a transforming role of KSHV-cyclin has yet to be established, we have found KSHV-cyclin transcripts in three out of three primary Kaposi’s sarcoma biopsies of different aetiology and in a primary effusion lymphoma, indicating a role for KSHV-cyclin in these tumours. The ability of KSHV-cyclin to overcome RB-mediated cell cycle arrest in SAOS2 cells provides direct evidence for a mechanism by which this virus could contribute to tumour development.

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