Restriction of human herpesvirus 6B replication by p53

Bodil Øster, Emil Kofod-Olsen, Bettina Bundgaard and Per Høllsberg

Institute of Medical Microbiology and Immunology, University of Aarhus, Aarhus, Denmark

Human herpesvirus 6B (HHV-6B) induces significant accumulation of p53 in both the nucleus and cytoplasm during infection. Activation of p53 by DNA damage is known to induce either growth arrest or apoptosis; nevertheless, HHV-6B-infected cells are arrested in their cell cycle independently of p53, and only a minor fraction of the infected cells undergoes apoptosis. Using pifithrin-α, a p53 inhibitor, and p53-null cells, this study showed that infected epithelial cells accumulated viral transcripts and proteins to a significantly higher degree in the absence of active p53. Moreover, HHV-6B-induced cytopathic effects were greatly enhanced in the absence of p53. This suggests that, in epithelial cells, some of the functions of p53 leading to cell-cycle arrest and apoptosis are restrained by HHV-6B infection, whereas other cellular defences, causing inhibition of virus transcription, are partially retained.

INTRODUCTION

The betaherpesvirus human herpesvirus 6B (HHV-6B) is the cause of one of the most common childhood diseases in the Western world, exanthem subitum (Yamanishi et al., 1988). HHV-6B establishes latency in its host during the first years of life (Braun et al., 1988). HHV-6B establishes latency in its host during the first years of life (Braun et al., 1988), and reactivation may occur throughout life, with excretion of HHV-6B in the saliva (Boutolleau et al., 2006). However, reactivation during conditions of immunosuppression may have severe consequences, such as encephalitis (Braun et al., 1997).

HHV-6B and the closely related virus HHV-6A both use the ubiquitously expressed surface protein CD46 as a cellular receptor. However, additional receptors for both HHV-6A and -6B are likely, as the two variants have different tropisms, and also appear to have distinct epidemiological and biological properties (Braun et al., 1997; Donati et al., 2005; Hall et al., 1998; Pedersen & Høllsberg, 2006). Upon infection, HHV-6B blocks host-cell DNA replication (Di Luca et al., 1990) within the first 12 h in T cells (Øster et al., 2005). This parallels an increase in protein synthesis (Black et al., 1992; Di Luca et al., 1990), consistent with a block in the G1/S phase of the cell cycle (Øster et al., 2005). In addition, there may be a block in the G2 phase (De Bolle et al., 2004; Øster et al., 2005). Importantly, the reduction in cellular DNA synthesis in HHV-6B-infected cells is not caused by induction of necrosis or apoptosis (Øster et al., 2005; Takemoto et al., 2004).

Prior to the cell-cycle arrest, a significant increase in the level of p53 protein can be detected in HHV-6B-infected cells (De Bolle et al., 2004; Øster et al., 2005; Takemoto et al., 2004). As a transcription factor, p53 may induce or repress a variety of cellular genes that are important for biological processes, such as induction of cell-cycle arrest, apoptosis and DNA repair (Vogelstein et al., 2000). A recent map of p53-binding sites in the whole human genome has defined at least 542 binding loci, suggesting a complex regulation of p53-induced genes (Wei et al., 2006). The increased level of p53 protein in HHV-6B-infected cells is not paralleled by an increase in p53 mRNA and is therefore likely to be the result of increased stabilization (Øster et al., 2005; Takemoto et al., 2004). The regulation of p53 protein levels is in part controlled by the ubiquitin E3 ligase Mdm2, which transfers ubiquitin to p53, thereby promoting its nuclear export and degradation by the ubiquitin-dependent proteasomal system (Haupt et al., 1997; Kubbhat et al., 1997). Supporting the notion that p53 may be stabilized, a reduced p53 ubiquitination after HHV-6B infection has been reported (Takemoto et al., 2004). Similar results are seen for other herpesviruses in that human cytomegalovirus (HCMV) stabilizes p53 without increasing its synthesis (Fortunato & Spector, 1998) and ICP0 from herpes simplex virus type 1 (HSV-1) increases the level of p53, independent of p53 transcription (Hobbs & DeLuca, 1999). The accumulation of p53 in HHV-6B-infected cells can be identified in both cytoplasmic and nuclear fractions. Indeed, nuclear p53 is phosphorylated at Ser-15 and Ser-20 and binds to its consensus DNA sequence. Although these findings are consistent with increased p53 transcriptional activity, paradoxically neither p21 nor PUMA appear to be induced in HHV-6B-infected cells (Øster et al., 2005).

HHV-6A and -6B encode proteins that interact with p53. The protein product of the HHV-6A gene DR7 binds directly to p53 and has malignant transforming activity in...
NIH3T3 cells. Moreover, cells expressing DR7 protein produce fibrosarcoma when injected into nude mice (Kashanchi et al., 1997). A similar open reading frame is present in HHV-6B, but it is unknown whether the HHV-6B-encoded DR7 protein exhibits similar functions. Furthermore, the U14-encoded tegument protein from HHV-6A and -6B may associate with p53. Interestingly, the U14 protein may tether p53 to the viral particle (Takekoto et al., 2005), although the function of this interaction has not been established. Therefore, it also remains to be investigated whether U14 may inactivate p53 during infection.

We have demonstrated previously that p53 is dispensable for HHV-6B-induced inhibition of DNA synthesis, and that this inhibition is more pronounced in the absence of p53 (Öster et al., 2006). Here, we report that p53 restricts the production of HHV-6B mRNAs and proteins, as well as the cytopathic effects (CPE) of the virus. This suggests that some of the key functions of p53 leading to cell-cycle arrest and apoptosis are restrained by HHV-6B infection, whereas other cellular defences, causing inhibition of virus transcription, are partially retained.

**METHODS**

**Cells.** The human colon cancer epithelial cell lines HCT116 wt and HCT116 p53−/− (kind gifts from B. Vogelstein and K. W. Kinzler, Johns Hopkins University, Baltimore, MD, USA), were grown in McCoy’s medium supplemented with extra glutamine (0.146 g l−1), 10 mM HEPES, streptomycin (0.2 g l−1), penicillin (0.2 IU l−1) and 10% heat-inactivated fetal bovine serum (FBS). MOLT3 cells were grown in Iscove’s modified Dulbecco’s medium (IMDM) supplemented with 10% heat-inactivated FBS, glutamine (0.292 g l−1), 10 mM HEPES, streptomycin (0.2 g l−1) and penicillin (0.2 IU l−1).

**Virus infections.** The MOLT3 T-cell line was used for propagation of HHV-6B (strain PL1) in IMDM supplemented as described above. Cells were infected at a concentration of 2 × 106 cells ml−1. After 3 days of infection, half of the cells and medium were frozen separately at −70 °C and replaced with an equal number of uninfected MOLT3 cells, resuspended in a volume of fresh IMDM corresponding to one-third of the removed volume. After 5 h of infection, additional IMDM was added to maintain an unchanged volume for continuous virus propagation. The procedure of collecting cells and supernatants at −70 °C and re-infecting the cell culture was continued for up to 6 weeks. Cells were followed visually for CPE, and mock-infected cells was replaced with fresh medium at the time of infection.

**Detection of CPE.** Photographs of MOLT3, HCT116 wt and HCT116 p53−/− cells in the presence or absence of HHV-6B infection were obtained using an Olympus IX71 microscope with a Leica DFC350F camera attached. In each well of a six-well plate, 2.5 × 106 cells were infected in 800 µl McCoy’s medium containing HHV-6B diluted 1:1. Photographs of HHV-6B- and mock-infected cells were taken at 48, 72 and 96 h post-infection (p.i.).

**Confocal microscopy.** HCT116 wt cells, infected with HHV-6B diluted 1:10, were grown on poly-l-lysine-coated cover slips for 48 h p.i. and fixed in 4% formalin in PBS (pH 7.5). Cells were washed twice in PBS, blocked in 5% BSA in PBS and permeabilized in 0.2% Triton X-100 in PBS. The nuclear viral protein p41 was visualized using a mAb against p41 (diluted 1:200; Abi/Advanced Biotechnologies) and a secondary F(ab)2 antibody conjugated to Alexa Fluor 488 (diluted 1:200). The nucleus was visualized using the DNA dye TO-PRO-3 iodide 642/661 (diluted 1:1000; Invitrogen). Images were obtained using the 488 nm line of a multi-line argon laser and the 633 nm line of a helium-neon laser on an upright LEICA TCS SL confocal microscope with a ×40 oil-immersion objective.

**Cell lysis and Western blotting.** Cells were lysed for 30 min in 1× lysis buffer (Cell Signaling Technology) supplemented with 1 mM PMSF, 5 mM NaF and Complete Mini Protease Inhibitor (Roche Diagnostics) at a concentration recommended by the manufacturer. Lyssates were centrifuged at 2600 g for 5 min, followed by 20 000 g for 10 min, and whole-cell extracts were immediately frozen at −70 °C. Nuclear and cytoplasmic extracts were obtained using a nuclear extract kit (Active Motif) according to the manufacturer’s instructions. In brief, nuclear and cytoplasmic extracts were separated by first collecting the cells in ice-cold PBS in the presence of phosphatase inhibitors. Next, cells were resuspended in a hypotonic buffer to swell the cell membrane and make it fragile. NP-40 was added to make the cells leak cytoplasmic proteins into the supernatant. At this step, the cytoplasmic fraction was collected. Nuclear proteins were extracted from the nuclei in a lysis buffer provided by the manufacturer, in the presence of a protease inhibitor cocktail. Proteins were separated in XT Criterion 10 % gels (Bio-Rad) using XT MES running buffer (Bio-Rad) for 1 h at 175 V and subsequently transferred to nitrocellulose membranes for 1 h at 300 mA. Two HHV-6B nuclear proteins were visualized using mAbs against p41 (Abi/Advanced Biotechnologies) and anti-herpes virus type 6 mAb (clone 7C7; Argene), both diluted 1:2000. The secondary antibodies, horseradish peroxidase-conjugated rabbit anti-mouse or pig anti-rabbit antibody (Dako), were diluted 1:2000. All one day prior to infection, cells were washed in 1× PBS and trypsinized in 0.05% trypsin in PBS with 0.54 mM EDTA for 5 min using 3 ml trypsin solution per 75 cm² flask. Cells were diluted to 10 ml in McCoy’s medium and counted. Between 0.5 × 10⁹ and 1.0 × 10⁹ cells at a concentration of 1 × 10⁶ cells ml−1 were transferred to each well of 24-well plates. At the time of infection, the medium was removed and cells were infected using HHV-6B diluted 1:10 for whole-cell extracts or 1:3 for RNA isolation. The medium from mock-infected cells was replaced with fresh medium at the time of infection.

**Inactivation and neutralization of virus.** Virus was UV-inactivated for 20 min in a 24-well plate 5 cm from a 15 W UV-C lamp while kept on ice. Antibody-mediated neutralization of virus was achieved by incubation with an anti-gH monoclonal antibody (mAb) (a kind gift from G. Campadelli-Fiume, Bologna, Italy). [3H]Thymidine incorporation was used to measure anti-gH-mediated neutralization as described previously (Öster et al., 2006). HHV-6B pre-incubated with anti-gH mAb for 20 min at room temperature was used to infect HCT116 wt cells. Cells were incubated for 42 h. The last 18 h of incubation took place in the presence of 1 μCi (37 kBq) methyl-[3H]thymidine (Amersham Biosciences) per well. Plates were frozen at −20 °C until harvesting.

**Images were obtained using the 488 nm line of a multi-line argon laser and the 633 nm line of a helium-neon laser on an upright LEICA TCS SL confocal microscope with a ×40 oil-immersion objective.**
antibodies were diluted in 5% skimmed milk in TBS with 0.1% Tween 20.

Pifithrin-α treatment. Simultaneously with infection of 1 × 10⁶ HCT116 wt cells with 250 μl HHV-6B (diluted 1:3), pifithrin-α [2-(2-imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)-1-p-tolyethane hydrobromide; Sigma-Aldrich] was added to a final concentration of 20 μM to infected and mock-infected cells (Komarov et al., 1999).

RNA purification. For RNA isolation, 1 × 10⁶ cells were washed in PBS and total RNA was isolated at room temperature using a High Pure RNA isolation kit (Roche Diagnostics) as described previously (Øster & Hollsberg, 2002). RNA was eluted in nuclease-free, sterile, double-distilled H₂O in a total volume of 70 μl. Total RNA concentrations were determined in duplicate by absorbance measurements (GeneQuant II RNA/DNA Calculator; Pharmacia Biotech).

RT-PCR. First-strand cDNA synthesis was performed on 400 ng total RNA mixed with 50 pmol random hexamer primers (Applied Biosystems), denatured at 65°C for 10 min. After addition of 1 × reverse transcriptase buffer, dNTP mix, disodium salt solution containing dATP, dCTP, dGTP and dTTP at final concentrations of 1 mM, 20 U RNase inhibitor and 2 U Transcriptor Reverse transcriptase (all from Roche Diagnostics), the RNA was incubated for 10 min at 26°C and then reverse transcribed for 30 min at 55°C. The reaction was stopped by raising the temperature to 85°C for 5 min. The cDNA was diluted to 40 μl with double-distilled H₂O. To avoid sample-to-sample contamination, separate laboratories were used for RNA purification, PCR set-up and template addition.

PCR amplification. Primer sequences for PCR amplification of HHV-6B genes (U7, U27, U37, U73, U81 and U86) have been published previously (Øster & Hollsberg, 2002). Amplification of cDNA was performed in duplicate by real-time PCR on a LightCycler instrument (Roche Diagnostics) in the presence of 2 μl cDNA, 3.5 mM MgCl₂, 0.5 μM primers and 5 μl QuantiTect SYBR Green (Qiagen) in a final volume of 10 μl. Each run included a positive control sample for the β-actin gene and negative controls for each primer pair either with no cDNA added or with cDNA from uninfected cells. All the reactions were carried out under the same conditions with an initial denaturation step at 95°C for 10 min, followed by 45 cycles of 95°C for 15 s, 62°C for 20 s and 72°C for 15 s. Melting curves were performed with one cycle at 95°C for 0 s, 72°C for 15 s and 99°C for 0 s.

p53 transfection. A p53-encoding vector (a kind gift from T. Hupp, Cancer Research UK, Edinburgh, UK) was transfected into HCT116 p53⁻/⁻ cells using Nucleofector (Amaxa, Program D-32) according to the manufacturer’s protocol. Control cells were transfected with transfection reagents without p53 vector. After 24 h of incubation, the transfected cells were infected with HHV-6B diluted 1:10 and incubated for an additional 24 h.

Reproducibility. All experiments were carried out with similar results in at least two independent set-ups.

RESULTS

HHV-6B induces CPE in MOLT3, HCT116 wt and HCT116 p53⁻/⁻ cells

HHV-6B-induced accumulation of p53 has been described in a variety of different cell types, including cord blood mononuclear cells, epithelial cells and the T-cell line MOLT3 (De Bolle et al., 2004; Øster et al., 2005, 2006; Takemoto et al., 2004). As a model for HHV-6B infection of epithelial cells, we used the human colon carcinoma cell line HCT116 wt and HCT116 p53⁻/⁻ cells that are genetically deficient for functional p53. In order to compare HHV-6B infection in HCT116 cells with MOLT3 cells, cells were followed visually for up to 4 days after infection. MOLT3, HCT116 wt and HCT116 p53⁻/⁻ cells infected with HHV-6B underwent syncytium formation (Fig. 1a). Cells infected with HHV-6B have been shown to display different degrees of CPE depending on the virus titre and cell type. At very high virus titres, cells may exhibit fusion from without at early time points after infection, independent of viral replication, whereas cells infected with lower virus titres need days to cause fusion as a consequence of de novo production of viral glycoproteins (Pedersen et al., 2006). These consequences are virus-specific, as neutralization of viral particles with an anti-gH mAb prevents early fusion (Pedersen et al., 2006) and HHV-6B-induced inhibition of [³H]thymidine incorporation (Fig. 1b). At 2 days p.i., large syncytia could be detected in p53⁻/⁻ cells, whereas syncytium formation in wild-type cells was much less pronounced at this time. At 3 and 4 days p.i., the syncytia in wt cells had increased further in size and quantity, whereas the p53⁻/⁻ culture appeared to have transformed into large aggregates with many dead cells. These observations suggested that p53 was restricting HHV-6B replication. UV-inactivated virus failed to induce CPE in any of the three cell types, indicating that virus replication is needed for the development of CPE in these cells (Fig. 1a).

In addition to cellular enlargement, almost all HCT116 wt cells stained positive for the viral U27-encoded nuclear protein p41 at 48 h p.i. (Fig. 1c), indicating that the vast majority of HCT116 wt cells were infected. Infected p53⁻/⁻ cells formed syncytia at early time points (Fig. 1a) and detached from the cover slips, rendering quantification of infection in p53⁻/⁻ cells by confocal microscopy impossible.

Using mAbs recognizing a 116 kDa nuclear protein (mAb 7C7) and the nuclear p41 protein, we then used Western blotting to examine whether UV-inactivated virus was capable of inducing p53 accumulation at 24 h p.i., and whether the two nuclear virus proteins could be detected following UV-inactivation of viral replication. Cells treated with UV-inactivated virus did not accumulate p53 or express the two nuclear virus proteins (Fig. 1d; results not shown for 115 kDa protein), demonstrating that de novo virus-encoded gene expression is required for HHV-6B-induced p53 accumulation and accumulation of p41 HHV-6B protein in HCT116 cells.

Pifithrin-α enhances the level of HHV-6B gene transcripts

Despite the significant accumulation of p53 in HHV-6B-infected cells, its functional role remains unclear. In order to investigate whether p53 affects HHV-6B replication, HCT116 wt cells were infected in the presence or absence...
Restriction of HHV-6B infection by p53

Fig. 1. HHV-6B requires virus transcription for fusion from within and accumulation of viral nuclear proteins. (a) MOLT3, HCT116 wt and HCT116 p53<sup>−/−</sup> cells form syncytia following HHV-6B infection. Cells lacking p53 formed large and more profound syncytia at earlier time points than wt cells. HHV-6B-infected MOLT3, HCT116 wt and HCT116 p53<sup>−/−</sup> cells were followed for 4 days p.i. and photographs were taken at 48, 72 and 96 h p.i. (b) The percentage proliferation was determined by [³H]thymidine incorporation in HHV-6B-infected HCT116 wt cells divided by the incorporation in mock-infected cells. HHV-6B was neutralized in the presence of anti-gH mAb diluted 1:1000, 1:2000, 1:4000 or 1:16000. (c) HCT116 wt cells were infected for 48 h with HHV-6B (diluted 1:10) and stained with anti-p41 mAb and an Alexa Fluor 488-conjugated secondary antibody. DNA nuclear staining was performed with TO-PRO-3 and the fraction of infected cells was determined by immunofluorescence microscopy. (d) Western blotting with anti-p53, anti-p41 (recognizing the U27 nuclear protein product p41) and anti-GAPDH antibodies of whole-cell extracts from MOLT3, HCT116 wt and HCT116 p53<sup>−/−</sup> cells infected for 24 h with HHV-6B (diluted 1:10).

of the small-molecule inhibitor of p53, pifithrin-α (Komarov et al., 1999). HHV-6B gene transcripts were detected by real-time PCR using primers specific for the HHV-6B genes U7, U27 and U73 (Öster & Höllsberg, 2002) and the constitutively expressed cellular β-actin gene. To compare the levels of viral gene transcripts, data were
normalized to β-actin and presented as the fold difference between treated and untreated, infected cells. Cells infected in the presence of pifithrin-α expressed significantly more of the viral transcripts from U7, U27 and U73 at 4 h p.i. compared with cells infected in the absence of pifithrin-α (Fig. 2). No transcripts could be detected in uninfected cells or in no-template controls. Thus, these observations suggest that p53 suppresses HHV-6B gene transcription.

**The level of HHV-6B gene transcription is enhanced in p53−/− cells**

Although inhibition of p53 by pifithrin-α in HCT116 wt cells enhanced the level of HHV-6B gene transcripts, we could not entirely exclude the possibility of p53-independent effects of pifithrin-α. To further address the role of p53 in HHV-6B replication, we made use of HCT116 cells in which the p53 gene was disrupted by homologous recombination (Fig. 3a) (Bunz et al., 1998). The level of HHV-6B gene transcripts was compared between HHV-6B-infected HCT116 p53−/− and HCT116 wt cells at 4 and 8 h p.i. (Fig. 3). Four of the investigated genes, the immediately-early (IE) genes U81 and U86 and the early (E) genes U7 and U73, demonstrated higher levels of transcripts in p53−/− cells at 4 h p.i., whereas the two late (L) genes U23 and U27 seemed to be unaffected at this time point. However, by 8 h p.i., all six of the investigated genes had higher levels of gene transcripts in the p53−/− cells compared with the wt cells. This further substantiated the involvement of p53 as a limiting factor in HHV-6B gene transcription.

**Expression of p53 restricts HHV-6B protein level**

We then examined by Western blotting whether p53 was able to restrict HHV-6B protein levels. Whole-cell extracts were obtained from HCT116 wt and p53−/− cells infected for 5 and 48 h. In agreement with the transcription data for U27 (Figs 2 and 3), p41 accumulated to a significantly higher level in p53−/− cells compared with wt cells at 48 h p.i. The HHV-6B protein p41 could not be detected at 5 h p.i. in p53−/−, HCT116 wt (Fig. 4a) or MOLT3 cells (Fig. 4b), indicating that accumulation of p41 as well as the
Overexpression of p53 in HCT116 wt and p53

Fig. 4. Accumulation of viral proteins during HHV-6B infection is restricted by p53. (a) Western blotting of whole-cell extracts from HCT116 wt and p53−/− cells infected with HHV-6B for 5 or 48 h was probed with anti-p41 mAb recognizing the U27 nuclear protein product p41. (b) Western blotting of whole-cell extracts from MOLT3 cells infected with HHV-6B for 5, 24 and 48 h. Proteins were detected with antibodies against p53, GAPDH and viral nuclear proteins using anti-p41 mAb and anti-human herpesvirus 6 mAb 7C7 recognizing the 116 kDa nuclear protein. (c) Overexpression of p53 in HCT116 wt and p53−/− cells. HCT116 wt and p53−/− control cells were treated with transfection reagents only, with no p53 vector. Proteins were detected by Western blotting using an anti-GAPDH antibody and mAb 7C7 recognizing the 116 kDa viral nuclear protein.

116 kDa nuclear protein, visualized with mAb 7C7 in MOLT3 cells, was the result of de novo protein synthesis. Overexpression of p53 in HCT116 wt and p53−/− cells for 24 h prior to HHV-6B infection for 24 h reduced the level of the 116 kDa nuclear protein compared with cells treated with transfection reagents only (Fig. 4c). Accumulation of the viral proteins therefore seemed to be significantly inhibited in the presence of p53.

DISCUSSION

The transcription factor p53 regulates several cellular functions and is known to induce either cell-cycle arrest, through a pathway involving the Cdk inhibitor p21, or apoptotic cell death by transcriptional activation of a different set of genes, including PUMA (Vogelstein et al., 2000). It has been reported previously that HHV-6B induces p53 accumulation and cell-cycle arrest in different cell types, in the absence of significant apoptosis (De Bolle et al., 2004; Øster et al., 2005; Takemoto et al., 2004). Nevertheless, p53 does not seem to induce apoptosis and is not involved in the observed cell-cycle arrest (Øster et al., 2006).

To address the role of p53 during HHV-6B infection, we first attempted to block p53 transcriptional activation using pifithrin-α (Komarov et al., 1999), and then used HCT116 cells disrupted in the p53 gene by homologous recombination (Bunz et al., 1998). Both experiments indicated that the levels of HHV-6B gene transcripts were increased in the absence of p53 activity. This was further confirmed by analysis of two different HHV-6B-encoded nuclear proteins that accumulated in the p53−/− cells faster and to a higher level than in the wt cells. Overexpression of p53 in HCT116 wt and p53−/− cells further supported the inhibitory effect of p53 on HHV-6B infection, as p53 transfection of both wt and p53−/− cells reduced expression of the 116 kDa HHV-6B nuclear protein. Together, these experiments indicate that p53 restricts the replication of HHV-6B.

Accumulation of p53 in the absence of p53-dependent growth arrest or apoptosis suggests that the virus may inactivate p53 or at least some of the p53 functions. Several DNA viruses have been shown to affect the stabilization and transcriptional activity of p53. HCMV seems to require p53 for its replication in a permissive fibroblast cell line, as p53−/− cells delay and decrease the accumulation of infectious viral particles (Casavant et al., 2006). This is in contrast to our findings, and may reveal different roles of p53 during productive and non-productive infections. HSV-1 stabilizes p53 by an unidentified mechanism that requires IE gene expression but occurs independently of viral DNA replication (Boutell & Everett, 2004). The Kaposi’s sarcoma-associated herpesvirus interferon regulatory factor 1 inhibits the transcriptional activity of p53 by interacting with the central DNA-binding domain of p53 (Shin et al., 2006). Adenovirus represses the transcriptional activation and growth-suppression functions of p53 via binding of adenovirus E1B 55K protein to p53 (Martin & Berk, 1998), and hepatitis B virus inhibits p53 sequence-specific DNA binding and transcriptional activity via binding of hepatitis B virus X protein to p53 (Wang et al., 1994). The mechanism by which HHV-6B interferes with p53 remains unclear, although two virally encoded p53-binding proteins have been identified. In HHV-6A and -6B, the U14 protein has been shown to bind p53 (Takemoto et al., 2005), and HHV-6A also encodes a p53-binding protein from the DR7 gene (Kashanchi et al., 1997). A protein product from the DR7 homologue in HHV-6B has not yet been demonstrated experimentally.

There may be several reasons why some viruses inactivate p53. Inactivation of p53 may be a step towards...
circumventing a potential p53-mediated cellular growth restriction, thereby facilitating virus replication (Shin et al., 2006). This raises the question: why does virus then induce cell-cycle arrest by a p53-independent method? Virus infection is one of the cellular stress factors that can potentially activate p53 (Takaoka et al., 2003). Activation of p53 can have several consequences other than cell-cycle arrest, including apoptosis, which would be meaningless for HHV-6B to induce as it is a virus that needs 72 h to complete its life cycle. It is therefore plausible that HHV-6B has developed mechanisms to inhibit apoptosis, as has also been shown for HCMV (Mocarski, 2002), and inhibition of p53 may just be one of such mechanisms.

How p53 may restrict virus replication in HCT116 cells is unknown. The most straightforward explanation would be a negative regulatory role of p53 on viral promoters. Indeed, IE proteins and p53 have been shown to mediate a negative regulatory function on the HCMV UL94 late promoter (Wing et al., 1998). Whether a similar role of p53 is responsible for the restriction of HHV-6B gene expression in HCT116 cells remains to be explored.

In summary, our results demonstrate that p53 restricts HHV-6B replication without exerting its key cellular functions such as growth arrest and apoptosis. We speculate that the restrictions in p53 functions are caused by HHV-6B-encoded proteins. If this is correct, it raises a number of interesting issues to pursue: the inactivation of p53 and lack of apoptosis may make the cell more susceptible to mutations. However, the virus-induced growth arrest would inhibit the development of malignant cells. Thus, it becomes interesting to differentiate the mechanisms involved in p53 inactivation and growth arrest in order to examine whether these mechanisms always co-exist or whether chronic infection with HHV-6B under specific circumstances may allow cell proliferation in the presence of p53 inactivation.

ACKNOWLEDGEMENTS

We thank K. W. Kinzler and B. Vogelstein for HCT116 p53−/− cells, T. Hupp for the p53 plasmid and G. Campadelli-Fiume for the anti-gH mAb. This work was supported by grants from Flemming Petersen’s Foundation, The Foundation of 17.12.1981, The Lundbeck Foundation, The University of Aarhus Research Foundation and The Danish Medical Research Council.

REFERENCES


