Protein tyrosine phosphatase H1 is a target of the E6 oncoprotein of high-risk genital human papillomaviruses

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The E6 proteins of high-risk genital human papillomaviruses (HPV), such as HPV types 16 and 18, possess a conserved C-terminal PDZ-binding motif, which mediates interaction with some cellular PDZ domain proteins. The binding of E6 usually results in their ubiquitin-mediated degradation. The ability of E6 to bind to PDZ domain proteins correlates with the oncogenic potential. Using a yeast two-hybrid system, GST pull-down experiments and coimmunoprecipitations, we identified the protein tyrosine phosphatase H1 (PTPH1/PTPN3) as a novel target of the PDZ-binding motif of E6 of HPV16 and 18. PTPH1 has been suggested to function as tumour suppressor protein, since mutational analysis revealed somatic mutations in PTPH1 in a minor fraction of various human tumours. We show here that HPV16 E6 accelerated the proteasome-mediated degradation of PTPH1, which required the binding of E6 to the cellular ubiquitin ligase E6-AP and to PTPH1. The endogenous levels of PTPH1 were particularly low in HPV-positive cervical carcinoma cell lines. The reintroduction of the E2 protein into the HPV16-positive cervical carcinoma cell line SiHa, known to lead to a sharp repression of E6 expression and to induce growth suppression, resulted in an increase of the amount of PTPH1. Our data suggest that reducing the level of PTPH1 may contribute to the oncogenic activity of high-risk genital E6 proteins.

INTRODUCTION

Human papillomaviruses (HPV) are small DNA tumour viruses infecting the basal cells of the skin or mucosa. The resulting tumours are initially benign, however, infections with some HPV types imply a high risk to undergo malignant conversion. A subset of mucosotropic HPVs, including HPV16 and 18, are associated with cervical cancer (Munoz, 2000). In these cancers, the expression of the two viral oncoproteins E6 and E7 is usually upregulated. E6 and E7 transform cells by interfering with cell-cycle regulation and counteracting apoptosis (reviewed by Longworth & Laimins, 2004; Münger et al., 2004). High-risk genital HPV E6 proteins target a number of cellular proteins for proteasome-mediated degradation, including p53. In complex with the cellular ubiquitin-protein isopeptide ligase E3, called E6-AP, E6 binds to the tumour suppressor p53, which leads to its ubiquitination and degradation (reviewed by Scheffner & Whitaker, 2003). The elimination of the function of p53 significantly contributes to E6-mediated oncogenesis by increasing the risk of accumulation of mutations. However, high-risk genital E6 have oncogenic activities independent of targeting p53 (Kiyono et al., 1997, 1998; Liu et al., 1999). High-risk genital E6 proteins bind to PDZ (PSD95/Dlg/ZO-1) domain proteins via four conserved C-terminal amino acids encoding a PDZ-binding motif (Songyang et al., 1997). E6 proteins reveal specificity for particular PDZ domains; however, the molecular basis for this specificity is unknown (Thomas et al., 2001). To date, several cellular PDZ domain proteins such as hDlg, hScribble, MUPP1 and MAGI-1-2-3 have been identified as targets of high-risk HPV E6. Most of these factors are prone to ubiquitin-mediated proteolysis (reviewed by Scheffner & Whitaker, 2003). The PDZ-binding motif of E6 proteins is essential for transformation of established rodent cell lines (Kiyono et al., 1997) and for the induction of epithelial hyperplasia in transgenic mice expressing HPV16 E6 (16E6) (Nguyen et al., 2003; Simonson et al., 2005), indicating that the interaction with and presumably the degradation of the PDZ domain proteins contributes to the oncogenic potential of E6. However, the biologically relevant targets involved in this process still have to be determined.

Phosphorylation of tyrosine residues controlled by protein tyrosine kinases (PTKs) and phosphatases (PTPs) is a
central feature of many cellular signalling pathways, including those affecting growth, differentiation, cell-cycle regulation, apoptosis and invasion (reviewed by Ostman et al., 2006). Although a variety of PTK genes have been directly linked to tumorigenesis through somatic activating mutations, only a few PTP genes have been implicated in cancer. A comprehensive mutational analysis of the PTP gene superfamiliy identified somatic mutations in six PTP genes in 26% of colorectal cancers and occasionally in other cancers. Expression of wild-type, but not mutated, tyrosine phosphatases inhibited growth of cancer cells, indicating that the tyrosine phosphatases are tumour suppressors. Among them was PTPH1, also named PTPN3 (Wang et al., 2004). PTPH1 belongs to the subfamily of the non-receptor PTPs and encodes within the N terminus a region with homology to a band 4.1 ezrin radixin moesin (FERM) domain, responsible for targeting proteins to the cytoskeleton–membrane interface. PTPH1 has a C-terminal catalytic phosphatase and one central PDZ domain (Arpin et al., 1994; Yang & Tonks, 1991; Zhang et al., 1995). Overexpression of PTPH1 resulted in growth inhibition of NIH3T3 cells, which may rely on the dephosphorylation of the cell-cycle regulator VCP (valosin-containing protein) (p97/CDC48), one of the few substrates of PTPH1 identified (Zhang et al., 1999).

Here, we report the identification of PTPH1 as a target protein of the PDZ-binding motif of 16E6 by a yeast two-hybrid system. We demonstrate that the interaction induced the degradation of PTPH1 in vivo and in vitro, which required the binding of E6 to the ubiquitin ligase E6-AP as well. We thus confirm observations made by Jing et al. (2007), who identified PTPH1/PTPN3 as a target of the 16E6–E6-AP complex by a substrate trapping assay. Beyond that, we show that the levels of endogenous PTPH1 were particularly low in HPV-positive cervical cancer-derived cell lines that express E6. Overexpression of the HPV16-encoded E2 protein, a repressor of the expression of E6, led to increased PTPH1 concentrations in HPV16-positive SiHa cells, supporting the notion that 16E6 degrades endogenous PTPH1 in these cells. Moreover, the E6 protein of high-risk cutaneous HPV8 was still able to bind to PTPH1, implying that targeting of PTPH1 may be conserved among high-risk E6 proteins.

**METHODS**

**Cells and tissue culture.** HPV-negative C33A cells and HPV-positive cervical carcinoma-derived cell lines SiHa, Caski and HeLa were cultivated in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FCS and antibiotics. RTS3b cells (Purdie et al., 1993) were grown in E-medium. C33A and SiHa cells were transfected by the CaPO4 procedure as described previously.

**Expression vectors.** PCR products were cloned into the vector pGEX2T (Promega) to obtain GST–16E6-C and GST–16E6-N and into pcDNAFLAGG3.1 ( Müller-Schifflmann et al., 2006) to express 8E6, 16E6 and truncated versions thereof with an N-terminally fused FLAG epitope. pcDNA-FLAG16E6A123-126 and pcDNA-FLAG-16E6A47R were obtained by site-directed in vitro mutagenesis. The PTPH1-encoding insert isolated from the yeast plasmid pACT-PTPH1 was cloned into the vectors pCMV-Myc (Clontech), pcDNA3.1-FLAG and pGEX-5X (Promega). Myc–PTPH1APDZ, lacking the amino acids 504–598 was obtained by in vitro mutagenesis and HA–PTPH1 + Ex12 (haemagglutinin-conjugated) was described by Zhang et al. (1997). The ORF for FLAG–p53 was isolated from pBS-FLAG-p53 and cloned into pcDNA 3.1. Myc–NAP-1 expression vector has been described by Rehtanz et al. (2004) and expression vectors for HA–16E6, HA–E6-AP and HA–E6-APAMS have been described by Kuballa et al. (2007).

**Protein–protein interaction studies.** GST pull-down assays were performed according to Muller et al. (2002) and coimmunoprecipitations were described by Rehtanz et al. (2004). Endogenous PTPH1 was detected by Western blot with the monoclonal antibody 2-117, kindly provided by N. Tonks (Zhang et al., 1997, 1999). For immunoprecipitations to detect endogenous PTPH1, 1.2 mg of total cell extracts, prepared as described by Rehtanz et al. (2004), was incubated with 5 μl of the antibody 2-117 for 1 h at 4 °C; antigen–antibody complexes were collected with protein-A-Sepharose followed by Western blotting.

**In vitro and in vivo degradation assays.** Proteins were translated using the Promega TNT-coupled transcription/translation system in the presence of 35S-Cys and mixed in a ratio of 2:3 PTPH1 or p53 and the respective E6 protein. Reactions were incubated at 30 °C for the indicated time before analysis. In order to investigate the in vivo degradation, 15 μg C33A cells extracts, prepared 24 h after transfection, were subjected to Western blotting with the anti-Myc, anti-HA (both Roche Diagnostics) or the anti-p53 (DO-1; Santa Cruz Biotechnology) antibodies to detect the respective proteins.

**RESULTS**

**PTPH1 binds to 16E6**

To search for cellular factors targeted by the PDZ-binding motif of 16E6, we performed a classical yeast two-hybrid screen with the 12 most C-terminal amino acids of 16E6, including the PDZ-binding motif as bait and a cDNA library derived from the keratinocyte cell line HaCat. In addition to the cellular factors hScrib, hDlg and Tip-1, all known as targets of the PDZ-binding motif of 16E6 (Hampson et al., 2004; Kiyono et al., 1997, 1999). For coimmunoprecipitations to detect endogenous PTPH1, 1.2 mg of total cell extracts, prepared 24 h after transfection, were subjected to Western blotting with the anti-Myc, anti-HA (both Roche Diagnostics) or the anti-p53 (DO-1; Santa Cruz Biotechnology) antibodies to detect the respective proteins.
expression vectors encoding FLAG-tagged 16E6 and Myc-tagged PTPH1. When coexpressed with FLAG–16E6, Myc–PTPH1 was recovered in FLAG–16E6 immunoprecipitates. In contrast, immunoprecipitates from cells which did not express FLAG–16E6 did not contain PTPH1. In addition, FLAG–16E6 D123-126 was able to specifically precipitate Myc–PTPH1 (Fig. 1b). The PDZ domain is a specific type of protein–protein interaction module with a structurally well defined interaction ‘pocket’ that can be filled by a PDZ-motif ‘ligand’ (Dev, 2004). This mode of association seems to occur in the case of the interaction between 16E6 and PTPH1, since FLAG–16E6Δ123-126 failed to precipitate Myc–PTPH1Δ504-598, with a deletion of its PDZ domain (Fig. 1b). Moreover, GST–16E6-C specifically retained endogenous PTPH1 from extracts of primary keratinocytes (Fig. 1c). Taken together, 16E6 binds via its C-terminal PDZ-binding motif to the PDZ domain of PTPH1 in vitro and in vivo.

**Binding of E6 to PTPH1 enhances its degradation**

The interaction of 16E6 with its PDZ partners usually leads to their accelerated degradation (Kiyono et al., 1997; Massimi et al., 2004), although exceptions have been described (Hampson et al., 2004). To test whether 16E6 induces the degradation of PTPH1, in vitro degradation assays were performed by coincubating 35S-labelled 16E6 and PTPH1, both obtained by in vitro translation via a rabbit reticulocyte lysate, for various time points. As indicated in Fig. 2(a), the amount of PTPH1 gradually decreased with increasing incubation time only in the presence of 16E6. After 2 h, the PTPH1 specific band disappeared, demonstrating that 16E6 reduced the stability of PTPH1. The kinetic of the 16E6-mediated degradation of PTPH1 was comparable to that of p53 (Fig. 2a, lanes 7–12). The targeting of PTPH1 was essential for degradation, since the stability of 16E6-binding deficient...
PTPH1Δ504-598, with the deletion of the PDZ domain, was not affected after 3 h incubation with 16E6 (Fig. 2b).

To gain insights into the sequence requirements of 16E6, the capacity of different E6 mutants to degrade PTPH1 was analysed in vitro and in vivo. For in vitro degradation assays, C33A cells were cotransfected with expression vectors for Myc–PTPH1 and for the various 16E6 mutants, respectively. To exclude variations of the Myc–PTPH1 levels due to different transfection efficiencies, we included an expression vector for Myc-tagged nucleosome assembly protein-1 (NAP-1), which was not affected by 16E6, as internal control. As shown in Fig. 3(a, b), the binding of 16E6 to PTPH1 is a prerequisite to induce the degradation of PTPH1, since 16E6 to PTPH1 is a prerequisite to induce the degradation of PTPH1, independently of whether the ETQL was present or not (Fig. 3a, b). The proteasomal degradation of many cellular partner proteins bound by the PDZ-binding motif requires the complex formation of E6 with E6-AP, although other ubiquitin ligases may be involved as well, as suggested for hDlg (Pim et al., 2000). To address the question whether E6-AP is the ubiquitin ligase mediating the proteasomal degradation of PTPH1, we tested two known 16E6 mutants, differing in binding to E6-AP. The deletion of amino acids 123–126 abolished interaction with E6-AP, the degradation of p53 and conferred a defect in immortalization (Foster et al., 1994; Gewin et al., 2004; Kiyono et al., 1998; Klingelhoetz et al., 1996; Liu et al., 1999). This deletion eliminated the capacity of 16E6 to accelerate the degradation of PTPH1 (Fig. 3a, b). The second mutant we used was 16E6F47R, described by Nomine et al. (2006). The exchange of F to R at position 47 did not affect the binding to E6-AP, but eliminated the capacity to induce the degradation of p53 by a defect in recruiting p53 to the complex 16E6–E6-AP (Nomine et al., 2005, 2006; Zanier et al., 2005). This was analysed in the context of the additional mutation of the C in position 6 to S (Nomine et al., 2006). We observed that in the context of the wild-type 16E6, the mutation F47R abrogated the ability to degrade p53 as well (data not shown), but did not affect the degradation of PTPH1 in vitro and in vivo (Fig. 3a, b). Thus, there is a correlation between binding of 16E6 to E6-AP and the ability to induce the degradation of PTPH1. We also determined the effect of the proteasome inhibitor MG132 upon PTPH1 protein levels in the presence of 16E6 in transiently transfected C33A cells. PTPH1 was stabilized upon treatment with MG132, indicating that PTPH1 is normally degraded by the proteasome. In the presence of 16E6, a dramatic decrease of the level of PTPH1 could be observed, which was restored when the cells were cultured in the presence of the proteasome inhibitor (Fig. 3b, lanes 8–11), supporting the notion that the proteasome is involved in the 16E6-mediated degradation of PTPH1.

This was further confirmed by the use of the stable H1299-K3 cell line, in which E6-AP expression was stably suppressed by RNA-interference, as described by Kuballa et al. (2007). While parental H1299 cells efficiently supported the degradation of PTPH1 by 16E6, this was markedly reduced in H1299-K3 cells. The ability of 16E6 to degrade PTPH1 in H1299-K3 cells was restored after overexpression of an E6-AP protein that was resistant to the siRNA (small interfering RNA) used to abrogate expression of endogenous E6-AP (see Kuballa et al., 2007). The deletion of amino acids 378–395 of E6-AP (E6-APΔBS), encoding the E6-binding site, failed to rescue the degradation of PTPH1 (Fig. 3c). The differences in the amounts of PTPH1 protein were not due to different expression levels of 16E6, as shown by the Western blot in Fig. 3(c). Taken together, the proteasomal degradation of PTPH1 by 16E6 requires the binding of E6 to the ubiquitin ligase E6-AP as well as to PTPH1.
During our studies we realized that all 24 cDNAs for PTPH1, isolated by the initial yeast two-hybrid screen, lacked amino acids 334–379. This segment is located between the band 4.1-FERM homology region and the

Fig. 3. The degradation of PTPH1 requires the interaction between 16E6 and PTPH1 and involves the proteasome. (a) In vitro degradation assay with 35S-labelled, in vitro-translated PTPH1 and 16E6 or various mutants thereof. The presence of PTPH1 was analysed by autoradiography after overnight incubation of the proteins. (b) In vivo degradation assays. An expression vector for Myc–PTPH1 was cotransfected with expression vectors for FLAG–16E6 or various mutants of 16E6, as indicated in the figure (lanes 1–7). An expression vector for Myc–hNAP-1 was included as internal transfection control. Myc–PTPH1 and Myc–NAP-1 were detected by Western blot developed with the anti-Myc antibody. To analyse the expression of the various E6 proteins, extracts of the transfected C33A cells were incubated with FLAG affinity matrix and bound E6 proteins were detected by Western blot developed with the anti-FLAG antibody. In lanes 8–11, transfected cells, as indicated in the figure, were incubated in the presence of either 25 μM MG132 (lanes 8, 10) or solvent (lanes 9, 10) before harvesting and analysis as above. The percentage of PTPH1 was quantified. (c) Parental H1299 cells (lanes 6–10) or H1299-K3 cells (lanes 1–5), in which the expression of E6-AP had been stably suppressed by RNA interference (Kuballa et al., 2007), were transiently transfected with expression vectors for Myc–PTPH1, HA–16E6, HA–E6-AP wild-type (wt) or E6-APΔBS, with a deletion of the E6-binding site from amino acids 378–395 (Kuballa et al., 2007), in different combinations, as indicated in the figure. The presence of Myc–PTPH1, p53, HA–16E6 and HA–E6-AP in the extracts was analysed by Western blot developed with the anti-Myc, -HA or -p53 antibodies. The signals corresponding to Myc–PTPH1 were quantified and the ratios are given. (d) 16E6 also degrades PTPH1+Ex12. In vivo degradation assay with extracts from C33A cells transiently transfected with expression vectors for HA-tagged PTPH1+Ex12, HA–NAP-1 and FLAG–16E6. HA–PTPH1+Ex12 and HA–NAP-1 were detected by Western blot. The structure of PTPH1, with the positions of the region homologous to band 4.1, the PDZ domain and the phosphatase (PTPc) domain, as well as exon 12, is shown.
PDZ domain (for overview, see Fig. 3d), but does not exhibit structural similarity to any known protein motifs. The 45 amino acids lacking in all our clones are exactly encoded by exon 12 (data not shown), as revealed by a BLAST search. The mRNA for PTPH1 contains 24 exons. Thus, our cDNAs for PTPH1 presumably represent an alternative splice product. The full-length HA-tagged PTPH1, HA–PTPH1 + Ex12 (Zhang et al., 1997), was degraded by 16E6 as was wild-type (Fig. 3d), demonstrating that the amino acids encoded by exon 12 do not affect the interaction between 16E6 and PTPH1.

The targeting of PTPH1 is conserved among E6 proteins

As already mentioned, the PDZ-binding motif is conserved among E6 proteins of high-risk genital HPVs and, as expected, HPV18 E6 was able to induce the degradation of PTPH1 in vitro and in vivo (Fig. 4a). In contrast, high-risk cutaneous E6 proteins do not encode a C-terminal PDZ-binding motif, although possessing transforming activity as well, as shown in the case of the high-risk cutaneous HPV8 (Iftner et al., 1988; Kiyono et al., 1992). Moreover, HPV8 E6 (8E6) does not bind to and induce the degradation of p53 (Kiyono et al., 1992; Steger & Pfister, 1992). Surprisingly, 8E6 specifically precipitated coexpressed Myc–PTPH1 (Fig. 4b). While 8E6-N (from amino acid 1 to 93) interacted with PTPH1, 8E6-C (amino acids 94–155) failed to do so, indicating that the N-terminal moiety was responsible for the binding. In correlation, 8E6Δ132-136, deficient in interaction with the cellular coactivator p300 (Müller-Schiffmann et al., 2006), precipitated PTPH1 as well. Although the binding of C-terminal PDZ motifs to PDZ domains appears to be the typical mode of interaction, it has been described that PDZ domains can contact internal motifs as well (Hung & Sheng, 2002; Nourry et al., 2003). However, PTPH1Δ504-598, lacking the PDZ domain, still interacted with 8E6, as demonstrated in the coimmunoprecipitation shown in Fig. 4(b). Obviously, the interaction between PTPH1 and 8E6

![Fig. 4.](http://vir.sgmjournals.org)
does not involve the PDZ module, but requires other regions. It became obvious in Fig. 4(b), that the amount of Myc–PTPH1 was not reduced when 8E6 was coexpressed, suggesting that 8E6 could not induce the accelerated degradation of PTPH1. This was confirmed by the in vitro degradation assay shown in Fig. 4(c).

Reducing the intracellular amounts of E6 correlates with increased levels of endogenous PTPH1 in SiHa cells

The findings described until here imply that, in established HPV-positive cervical cell lines, PTPH1 protein levels should be low, due to its degradation by E6. To test this, we compared the amount of PTPH1 in HPV-positive cell lines and in HPV-negative cells by immunoprecipitation. Fig. 5(a) shows that less PTPH1 was precipitated from extracts of HPV16-positive cervical carcinoma-derived cell lines SiHa, Caski and HPV18-positive HeLa cells, compared with primary human epithelial keratinocytes (NHEK), C33A and RTS3b cells, which all are HPV negative. Moreover, HaCat cells revealed higher levels of PTPH1 as well, in accordance with previous observations (data not shown) (Zhang et al., 1997). Thus, the reduced amounts of PTPH1 in HPV-positive cervical cancer cell lines may be a result of its degradation by E6, expressed in these cell lines.

Integration of HPV into the host genome is an important step in cervical neoplastic progression and usually causes deletion or disruption of the ORF for the viral transcription factor E2, while the expression of the E6 and E7 genes is retained (reviewed by Longworth & Laimins, 2004; Münger et al., 2004). It is well established that overexpression of E2 from heterologous promoters in HPV-positive cervical carcinoma-derived cell lines represses the early HPV promoter, resulting in a sharp reduction in E6 and E7 expression (Desaintes et al., 1997; Goodwin & DiMaio, 2000; Goodwin et al., 1998; Thierry & Yaniv, 1987). In order to test whether this E2-mediated repression correlates with a change in the level of PTPH1 protein, we transfected SiHa cells with an expression vector for FLAG-tagged HPV16 E2 (16E2). The introduction of E2 into cervical carcinoma cell lines such as HeLa has been shown to result in growth arrest or apoptosis (Francis et al., 2000; Nishimura et al., 2000) (reviewed by Blachon & Demeret, 2003). In order to minimize these pathways, the transfected cells were enriched by selection with G418 for only 48 h. In accordance with previous observations, overexpression of E2 led to a significant reduction of the E6 mRNA level, as revealed by RT-PCR (data not shown). Compared to the empty vector, the amount of PTPH1 was significantly elevated in the extracts of SiHa cells transfected with the 16E2 vector. E2 per se had no effect on PTPH1, since the overexpression of 16E2 in C33A cells did not change the levels of PTPH1 (Fig. 5b). Thus, higher concentrations of PTPH1 in SiHa cells may be the result of reduced amounts of 16E6 due to E2-mediated repression of E6 transcription, which is in line with the model that 16E6 induces the accelerated degradation of endogenous PTPH1 in SiHa cells.

**DISCUSSION**

The binding to PDZ partner proteins by the E6 oncoproteins of high-risk genital HPVs contributes to the oncogenic potential. The deletion of the PDZ-binding motif impairs the ability of E6 to transform established rodent cell lines, to immortalize keratinocytes and to induce epithelial hyperplasia in transgenic mice (Kiyono et al., 1997, 1998; Liu et al., 1999; Mantovani & Banks, 2001; Nguyen et al., 2003; Simonson et al., 2005). This transforming activity inherent to the PDZ-binding motif presumably does not relate to one specific target but to a combined degradation of different PDZ domain proteins. Our results presented here suggest that PTPH1 is an

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**Fig. 5.** (a) HPV-positive cervical carcinoma-derived cell lines have low amounts of endogenous PTPH1. Immunoprecipitation: total cell extracts from the HPV16-positive cell lines SiHa and Caski and the HPV18-positive cell line HeLa, the HPV-negative cell lines C33A and RTS3b as well as from normal human epidermal keratinocytes (NHEK) were subjected to an immunoprecipitation with an antibody directed against PTPH1. The presence of PTPH1 was confirmed in a Western blot developed with the PTPH1 antibody. As control, the presence of actin in the extracts was determined by Western blot. (b) Introducing an expression vector for E2 into SiHa cells leads to increased level of PTPH1. SiHa or C33A cells were transfected with an expression vector for FLAG–16E2 or the empty pcDNA3.1-FLAG vector. Transfected cells were selected 24 h later with G418 for 48 h. Cell extracts were used to immunoprecipitate PTPH1 by the anti-PTPH1 antibody. As control, the presence of actin was determined with 15 μg of each extract.
PTPH1 as target of high-risk HPV E6 proteins

The specificity of the interaction was demonstrated by the use of recombinant PTPH1 and 16E6 proteins synthesized in different systems such as yeast, bacteria, in vitro translation via a rabbit reticulocyte lysate and in mammalian cells. Moreover, GST–16E6-C precipitated endogenous PTPH1, which underlines that the native protein can be targeted by 16E6 and that the binding is not restricted to recombinant PTPH1. In addition to the interaction of both proteins via their PDZ module, the proteasome-mediated degradation of PTPH1 by 16E6 presumably depends on the binding of E6 to E6-AP. This is supported by the use of an inhibitor of the proteasome, the analysis of the degradation capacity of various 16E6 mutants and by reconstitution experiments in H1299 cells clone K3, in which expression of endogenous E6-AP was reduced. The degradation of PTPH1 by 16E6 presumably depends on the binding of E6 to E6-AP. This interaction of both proteins via their PDZ module, the proteasome-mediated degradation of PTPH1 by 16E6 presumably depends on the binding of E6 to E6-AP. This is supported by the use of an inhibitor of the proteasome, the analysis of the degradation capacity of various 16E6 mutants and by reconstitution experiments in H1299 cells clone K3, in which expression of endogenous E6-AP was stably ablated by RNA interference. Our results are in line with the findings of Jing et al. (2007), demonstrating that the complex 16E6+E6-AP associates with and targets the degradation of PTPH1, although we cannot exclude that another ubiquitin ligase is involved, as suggested for the E6-mediated degradation of hDlg and the MAGI family of proteins (Grm & Banks, 2004; Pim et al., 2000, 2002).

The exon 12 of PTPH1, which was not present in the splice variant of PTPH1 which we have isolated from HaCat cells, has no effect on targeting of PTPH1 by 16E6. The fact that all 24 cDNA clones we have recovered from HaCat cells during the initial yeast two-hybrid screen lacked exon 12 indicates that this splice variant may be the major isoform of PTPH1 expressed in HaCat cells. A serine at position 359, located within the segment from amino acid 334 to 379 encoded by exon 12, has been shown to represent one of two binding sites to 14-3-3. The previous observation that in HaCat cells less PTPH1 was found in complex with 14-3-3 (Zhang et al., 1997) may rely on the presence of this splice variant in these cells.

The lower level of endogenous PTPH1 protein in HPV-positive cervical carcinoma-derived cell lines SiHa, Caski and HeLa, compared with HPV-negative cell lines and primary keratinocytes, correlates with the notion that the E6 oncoproteins expressed by the high-risk HPV types 16 and 18, present in these cell lines, induce the accelerated degradation of endogenous PTPH1. This assumption is consistent with the observation made by Jing et al. (2007), who found that the introduction of 16E6 into keratinocytes reduced the amount of endogenous PTPH1, and with our result, demonstrating that the PTPH1 level rose when E6 expression was repressed by the E2 transcription factor (Fig. 5b). PTPH1 upregulation due to overexpression of E2 did not result from E2-mediated apoptosis, since E2 was reported to induce apoptosis in the HPV-negative cervical cancer cell line C33A as well (Demeret et al., 2003), but has no effect on PTPH1 in these cells (see Fig. 5b). The integration of the HPV genome into a host chromosome resulting in loss of the E2 transcriptional repressor is regarded as one of the key events during HPV-induced carcinogenesis (reviewed by Longworth & Laimins, 2004; Münger et al., 2004). Upregulated E6/E7 expression confers the cells growth advantage (Pett et al., 2004, 2006). Reduced levels of PTPH1, as a consequence of high amounts of E6, may contribute to promote cell growth. This is supported by the observation that ectopically expressed PTPH1 disrupts cell-cycle progression in NIH3T3 cells, thus slowing down or arresting cell growth (Zhang et al., 1999), and inhibited growth of RTS3b cells as well (St. and G.S.; unpublished results). The specific targets mediating growth inhibition by PTPH1 are unknown. Although the cell-cycle regulator VCP (valosin-containing protein), tyrosine-phosphorylated in response to T-cell stimulation, was identified as a substrate of PTPH1 (Zhang et al., 1999), we did not detect any effect of 16E6 on the phosphorylation level of VCP. It seems feasible that other, not yet identified, substrates of PTPH1 may exist in human keratinocytes that mediate the growth regulatory effects of PTPH1. For instance, PTPH1 may be involved in phosphotyrosine-based signal transduction initiated by growth factor receptors. Jing et al. (2007) observed that siRNA-mediated targeting of PTPH1 reduced growth factor requirement of immortalized keratinocytes. This activity may be affected by E6, since E6-transduced immortalized keratinocytes revealed the same phenotype (Jing et al., 2007). Moreover, the N-terminal band 4.1-ERM domain is thought to target PTPH1 to cytoskeleton–membrane interfaces (reviewed by Ostman et al., 2006). This subcellular localization may determine, at least in part, its substrates. Thus, it is possible that the interaction of E6 with PTPH1 may play a role in the E6-mediated disruption of the epithelial tight junction and enhanced morphological transformation of immortalized human keratinocytes, which was reported to be dependent on the integrity of the PDZ-binding motif of E6 (reviewed by Mantovani & Banks, 2001; Watson et al., 2003).

An interaction with PTPH1 may be conserved among cutaneous high-risk E6 proteins as well, since 5E6 was able to bind to PTPH1 in GST pull-down experiments (data not shown), in addition to 8E6. As expected, this interaction does not involve the PDZ domain of PTPH1, as high-risk cutaneous E6 proteins do not encode a PDZ-binding motif. Our preliminary studies revealed that 8E6 binds to the C-terminal part of PTPH1 encoding the phosphate activity (D. Jordanovski, Institute of Virology, University of Cologne, and G.S.; data not shown). We are currently testing whether 8E6 will modulate the phosphate activity or substrate binding. Taken together, results suggest that targeting the putative tumour suppressor PTPH1 may be conserved among high-risk E6 proteins.

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