Ubiquitination and proteasome degradation of the E6 proteins of human papillomavirus types 11 and 18

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Human papillomaviruses (HPVs) are aetiological agents for genital warts and cervical cancer, the different pathologies of which are dependent on the type of HPV infection. Oncogenic HPV types associated with cancer are carcinogens by virtue of their oncogene products, which target key regulators of cell proliferation and apoptosis. The viral E6 protein from oncogenic HPV types plays a central role in carcinogenesis by exploiting the cellular proteasome degradation pathway in order to mediate the degradation of cellular proteins, most notably the prototype tumour suppressor protein p53. Much less is known about the cellular targets of E6 from the non-oncogenic HPV types associated with genital warts. It is also unclear what factors influence the level and stability of the viral E6 proteins in cells. This report demonstrates that both oncogenic and non-oncogenic HPV E6 proteins (from types 18 and 11, respectively) are ubiquitinated and targeted for degradation by the 26S proteasome. E6 domains required for the induction of p53 or DLG degradation, or E6AP binding, are not involved in proteasome-mediated degradation of HPV-18 E6. These results provide insight into the cellular modulation of E6 protein levels from both high-risk and low-risk HPV types.

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About 30 HPV types can infect the genital area resulting in benign or malignant pathologies (zur Hausen 1991, 1996; Munoz et al., 2003). Low-risk HPV types (for example types 6 and 11) are non-oncogenic and mainly associated with benign genital warts, whereas the high-risk HPV types (i.e. types 16 and 18) are considered oncogenic and are frequently associated with cervical intraepithelial neoplasia (CIN), which can lead to invasive cancers. The oncogenic potential of high-risk HPV types has been correlated with the in vitro transforming activities of the viral oncogenes E6 and E7 (Storey et al., 1988). E6 and E7 proteins from oncogenic HPV types represent carcinogens by virtue of their ability to inactivate key regulatory cellular proteins involved in cell proliferation and apoptosis. Viral E6 proteins from oncogenic HPV types are able to bind p53 (Werness et al., 1990) and mediate its degradation (Scheffner et al., 1990) in order to abrogate p53-mediated apoptosis in infected cells. Oncogenic HPV E6 proteins target p53 for degradation through association with a cellular ubiquitin ligase termed E6AP (Huibregtse et al., 1991, 1993) resulting in the ubiquitination of p53 and its subsequent degradation by the 26S proteasome. In addition to p53, E6 from oncogenic HPVs has also been reported to mediate the degradation of a number of other cellular proteins (reviewed by Scheffner & Whitaker, 2003) including the ubiquitin ligase E6AP (Kao et al., 2000), c-Myc (Gross-Mesilaty et al., 1998), Bak (Thomas & Banks, 1998), hDLG (Kiyono et al., 1997; Lee et al., 1997; Gardiol et al., 1999; Pim et al., 2000), MAGI-1 (Glausinger et al., 2000), hScrib (Nakagawa & Huibregtse, 2000) and MUPP-1 (Lee et al., 2000). E6 proteins from low-risk HPVs do not appear to target cellular proteins for proteasome-mediated degradation, and their cellular function is largely unknown. It is also unclear what mechanisms may influence the level of E6 in the cell. In this study, we investigated whether E6 proteins from HPV-11 (low-risk) and HPV-18 (high-risk) are themselves targets of

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ubiquitination and whether this influences the level of E6 in the cell.

In order to detect the HPV E6 protein in the cell, we initially constructed plasmids expressing fusion proteins linking a FLAG epitope tag to the N termini of HPV-11 and -18 E6. The FLAG-E6 expression vectors were created by cloning the CMV-FLAG polylinker region of the pFLAG-CMV-2 vector (Sigma) into the multiple cloning region of pCDNA3.1/Zeo (Invitrogen) and the E6 sequences were then cloned between the EcoRI and EcoRV restriction sites of the newly generated vector. To determine whether the N-terminal FLAG-tagged HPV-18 E6 protein was detectable and retained activity, we first generated a stable cell line expressing the HPV-18 E6 fusion protein. Human HT1080 cells were used because they express wild-type p53 protein. The FLAG-tagged HPV-18 E6 expressing and control HT1080 cells were exposed to 1 μM adriamycin to induce p53 expression and the level of p53 was determined by Western blot analysis. As shown in Fig. 1(A), high levels of p53 protein accumulated in HT1080 control cells after 4 h of adriamycin treatment, whereas little p53 was detected in cells expressing FLAG-18E6. Thus, the N-terminal FLAG-tagged 18E6 protein was functional in these cells with respect to mediating p53 degradation. It is noteworthy that although a C-terminal AU epitope-tagged HPV-18 E6 was also able to mediate p53 degradation, it was not able to mediate the degradation of the PDZ domain-containing MAGI-1 protein. In contrast, the N-terminal tagged HPV-18 E6 protein was able to mediate the degradation of both p53 and MAGI-1 (data not shown). Since PDZ domain-containing proteins such as MAGI-1 and DLG are major targets of E6 from oncogenic HPVs (Glausinger et al., 2000; Gardiol et al., 1999), we performed subsequent experiments using N-terminal tagged E6 proteins.

We next examined HPV-18 E6 levels following exposure of the cells to 40 μM of the proteasome inhibitor MG-132 (carbobenzoxy-L-leucyl-L-leucyl-L-leucinal) (Calbiochem) for 4 h. Untreated cells were incubated in the presence of an equal volume of the solvent, dimethyl sulfoxide (DMSO), and Western blot analysis was carried out using an anti-FLAG antibody (Sigma). As demonstrated in Fig. 1(B), proteasome inhibition with MG-132 resulted in a significant increase in the steady-state levels of FLAG-18E6, indicating that the HPV-18 E6 protein was targeted for proteasome-mediated degradation in these cells.

It was of interest to then compare MG-132-mediated stabilization of HPV-11 E6 to HPV-18 E6, and to determine whether HPV-18 E6 mutants unable to interact with E6AP

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**Fig. 1.** Effect of proteasome inhibitor on FLAG-18E6 levels in stably transfected HT1080 cells. (A) Stable HT1080 cells expressing FLAG-18E6 and control HT1080 cells were exposed to 1 μM adriamycin to stimulate p53 expression and cells were harvested at time 0, 1, 2 and 4 h in ice-cold lysis buffer containing 10 mM Tris/HCl pH 7.5, 50 mM KCl, 2 mM MgCl2, 1% Triton X-100, 3 μg aprotinin ml⁻¹, 1 μg pepstatin ml⁻¹, 1 μg leupeptin ml⁻¹, 1 mM DTT and 1 mM PMSF. SDS-PAGE of lysates was followed by immunoblotting with anti-p53 antibody. Note that the presence of FLAG-18E6 effectively removes p53 from the cells. (B) FLAG-18E6-expressing and control HT1080 cells were exposed to 40 μM MG-132 or DMSO (control) for 4 h. Cell lysates were prepared and immunoprecipitated with anti-FLAG M2 antibody. Immunoprecipitates were subjected to SDS-PAGE and Western blot analysis was performed using anti-FLAG antibody. The upper band representing IgG(L) shows equal loading of immunoprecipitates. (C) 10(1) cells were transfected with expression vectors for FLAG-11E6, FLAG-18E6 or the different FLAG-18E6 deletion mutants; 24 h post-transfection cells were incubated in the presence of 40 μM MG-132 or DMSO (control), and cell lysates were prepared by sonication and subjected to Western blot analysis using the anti-FLAG antibody. Equal loading was confirmed by non-specific bands, as shown in the lower panel.
or to mediate the degradation of p53 and DLG were targeted for proteasome degradation. A series of previously characterized HPV-18 E6 mutants were tagged with the FLAG epitope as detailed above and examined for their susceptibility to proteasome-mediated degradation. Following transfection of p53-null 10(1) cells with FLAG-tagged HPV-11 E6, HPV-18 E6, and various mutant HPV-18 E6 expression vectors, cells were incubated with and without MG-132, cell lysates were prepared 4 h following treatment, and Western blotting was performed using the anti-FLAG antibody as detailed above. As shown in Fig. 1(C), both FLAG-11E6 and FLAG-18E6 protein levels increased dramatically in the presence of the proteasome inhibitor. Likewise, all of the FLAG-18E6 mutants were stabilized by proteasome inhibition. Mutants unable to mediate p53 degradation included M2 (R10S, P11G) and ΔM (delta 28–31), while mutations inhibiting DLG degradation lie in the C-terminal half of 18E6 and included mutants ΔE (delta 101–204), ΔF (delta 113–117) and ΔG (delta126–130). Recent studies have shown ΔM to have a reduced ability to bind the ubiquitin ligase E6AP (Pim & Banks, 1999). Notably however, these mutants retained the 8 lysines of HPV-18 E6, with the exception of the ΔF mutant, which does not contain the 7th lysine of the E6 protein. Taken together, these data argue that both the high-risk and low-risk HPV E6 proteins were susceptible to proteasome degradation. Moreover, binding to E6AP or degradation of p53 and DLG was not necessary for proteasome-mediated degradation of HPV-18 E6.

In order to confirm the previous observation that proteasome inhibition stabilized E6, it was necessary to use an epitope tag other than FLAG in which we could determine whether the epitope tag itself was stabilized by proteasome inhibitor. Hence, constructs expressing E6 fusion proteins linking GFP to the N termini of HPV-11 and -18 E6 were generated. Briefly, E6 cDNA sequences were first amplified by PCR, followed by cloning into the pEGFP-C3 vector (Clontech) at the BglII and EcoRI restriction sites present within the oligonucleotide primers. The generation of in-frame fusions was confirmed by DNA sequencing. To determine whether the constructs expressed fusion proteins of the expected size, p53-null 10(1) cells were transfected with equal amounts of GFP, GFP-11E6, GFP-18E6 or p53-GFP expression plasmids and a lacZ expression vector. Total cell lysates were prepared by sonication and β-galactosidase activity was determined to confirm equal transfection efficiency. Equal amounts of protein were subjected to Western blot analysis with an anti-GFP antibody (Clontech). As shown in Fig. 2(A), native GFP and p53-GFP had the expected molecular masses of 27 kDa and 80 kDa respectively. Both GFP-11E6 and GFP-18E6 fusion proteins had the predicted molecular masses of 44–46 kDa. These data confirm the expression and detection of intact GFP-E6 fusion proteins of the predicted size in the transfected cells.

An in vivo degradation assay was carried out in transfected cells to determine whether the GFP-18E6 fusion protein retained the ability to mediate the degradation of p53. 10(1) cells, null for p53, were transfected with equal amounts of GFP-E6 or wild-type HPV E6 expression plasmids together with a p53 expression plasmid. A lacZ expression plasmid

![Fig. 2](image-url)

**Fig. 2.** Stabilization of GFP-11E6 and GFP-18E6 proteins in 10(1) cells following proteasome inhibition with MG-132. (A) Western blot analysis of GFP-E6 fusion proteins expressed in 10(1) cells 24 h post-transfection using an anti-GFP antibody, as indicated. The observed molecular masses for GFP (27 kDa), p53-GFP (80 kDa) and both GFP-E6 fusion proteins (44–46 kDa) were in agreement with the predicted molecular masses. To confirm equal loading, the Western blot was reprobed with an anti-tubulin antibody. (B) GFP-18E6-mediated degradation of p53 as determined by Western blot analysis 24 h post-transfection. A pool of anti-p53 monoclonal antibodies (pAb 1801, 1802 and 122) was used to monitor p53 levels in 10(1) cells co-transfected with plasmids expressing wild-type p53 plus a control plasmid GFP (No E6), GFP-18E6, wild-type HPV-18 E6, GFP-11E6, or wild-type HPV-11 E6, as indicated. A non-transfected cell lysate was also included (No p53). Transfection efficiencies were equalized by including a lacZ expression plasmid and measuring β-galactosidase activity in the cell lysates. (C) 10(1) cells were transfected with GFP, GFP-11E6 or GFP-18E6 expression vectors, and 24 h post-transfection cells were incubated in 40 μM MG-132 or DMSO (control) for 4 h. Cell lysates were prepared by sonication and subjected to Western blotting using anti-GFP antibody. Equal transfection efficiencies were confirmed by co-transfection of a lacZ expression vector and measuring β-galactosidase activity in the cell lysates.
was included and β-galactosidase activity was determined in the transfected cells to ensure equal levels of transfection efficiencies. After 24 h, cells were extracted and equal amounts of protein were analysed by Western blot. The levels of p53 protein were determined using a pool of anti-p53 monoclonal antibodies; pAb 1801, 1802 and 122 (Banks et al., 1986). As shown in Fig. 2(B), the GFP-18E6 fusion protein directed the degradation of p53, similar to wild-type HPV-18 E6, confirming that the GFP-18E6 fusion protein retained wild-type function with respect to its ability to mediate p53 degradation.

To address whether the increase in the steady-state levels of E6 upon proteasome inhibition was independent of the epitope tag, p53-null 10(1) cells transiently expressing GFP-E6 fusion proteins were treated with the proteasome inhibitor MG-132 at a concentration of 40 μM for 4 h and transfection efficiencies were determined as before. Cell lysates, prepared by sonication, were subjected to Western blot analysis as detailed above. As shown in Fig. 2(C), a significant increase in the steady-state levels of both GFP-11E6 and GFP-18E6 was observed following MG-132 treatment. In contrast, MG-132 treatment did not increase the steady-state level of GFP alone. These results support the previous data obtained with the FLAG-11E6 and FLAG-18E6 proteins in showing that proteasome inhibition results in the stabilization of E6 and this was independent of the epitope tag used to detect E6. Furthermore, lactacystin, another specific inhibitor of the proteasome, also mediated an increase in both GFP-11E6 and GFP-18E6 in transfected 10(1) cells (data not shown), further supporting the involvement of the proteasome in the degradation of HPV E6.

In order to ensure that the observed stabilization of GFP-11E6 and GFP-18E6 proteins in the presence of the proteasome inhibitor was due to the direct effect of inhibiting degradation by the proteasome, and not to secondary influences on E6 transcription or translation, we investigated the turn-over rate of the GFP-E6 proteins in the presence and absence of proteasome inhibitor. The protein half-life analysis for the GFP-E6 fusion proteins was carried out as described previously for GFP fusion proteins, by determining GFP fluorescence at various times in cells treated with the protein synthesis inhibitor cycloheximide (CHX) (Li et al., 1998). H1299 cells, null for p53, were transfected with GFP, GFP-11E6 or GFP-18E6 expression vectors, and cells were split into 60 mm dishes 9 h post-transfection in order to equalize transfection efficiencies. Twenty-four hours following transfection, cells were treated with either 100 μg CHX (Sigma) ml⁻¹, or 100 μg CHX ml⁻¹ with 50 μM MG-132 to inhibit proteasome function. Control cells were incubated in the presence of equal volumes of solvent. Cells were collected at 0, 2, 5 and 9 h following treatment, washed and resuspended in PBS with 1 % FBS, and fluorescence intensity was measured on a FACSscan (Becton Dickinson). An arbitrary fluorescence of 100 % was attributed to cells collected at the 0 h time-point. As shown in Fig. 3(A), control GFP-expressing cells in the absence of the CHX showed an increase in fluorescence over time, while CHX-treated cells showed a minor reduction in fluorescence levels. Cells treated with CHX and MG-132 showed fluorescence levels similar to CHX alone. These results are consistent with GFP not being targeted for proteasome-mediated degradation, resulting in the relatively long half-life of GFP. In comparison to native GFP, fusing the HPV-11 and -18 E6 proteins to GFP markedly reduced fluorescence in a time-dependent manner, which was clearly most evident in the presence of CHX where the estimated half-lives were approximately 7 h for both GFP-11E6 and GFP-18E6 proteins (Fig. 3B, C). However, inhibition of the proteasome with MG-132 in the CHX-treated cells restored the half-lives of GFP-11E6 and GFP-18E6 to levels similar to the control cells, confirming that proteasome inhibition increased the half-lives of the GFP-E6 fusion proteins (Fig. 3B, C). Thus, proteasome inhibition significantly reduced the degradation rates of both GFP-11E6 and GFP-18E6, to the same extent.

To confirm that the decrease in fluorescence of GFP-E6-expressing cells correlates to a reduction in protein levels, Western blot analysis was performed using the same experimental design as described above. Cell lysates were prepared by sonication at 0 and 5 h following treatment, and Western blotting was performed using an anti-GFP antibody (Fig. 3D). Quantifiable results were obtained using a VersaDoc Imaging system (Bio-Rad) and Quantity One software (Bio-Rad). GFP-11E6 protein levels decreased by approximately 42 % following 5 h of CHX treatment (Fig. 3F), which is identical to the reduction in fluorescence observed by flow cytometry (Fig. 3B); thus both methods provide the same estimate for GFP-11E6 half-life. Furthermore, proteasome inhibitor stabilized GFP-11E6 protein levels in cells treated with CHX similar to the stabilization observed by flow cytometry. Similarly, Western blot and FACS analysis also provided comparable estimates of GFP-18E6 half-life, with a decrease of 64 % and 47 % observed for protein levels (Fig. 3G) and cell fluorescence levels (Fig. 3C), respectively, following 5 h of CHX treatment. Proteasome inhibitor prevented the degradation of GFP-18E6 to a similar extent as observed by FACS. The Western blot results for control GFP also correlate well with those obtained by flow cytometry, and are in agreement with the long half-life of GFP. Taken together, both FACS and Western blot time-course analyses have demonstrated comparable turn-over rates for GFP-11 and -18 E6 proteins and similar stabilization effects of the proteasome inhibitor on the fluorescence and protein levels of both GFP-11 and -18 E6. The data presented in Fig. 3 therefore confirm that proteasome inhibition increased the half-lives of both GFP-11E6 and GFP-18E6.

Since the majority of cellular proteins targeted for degradation by the 26S proteasome are first covalently bound to a chain of ubiquitin proteins, it was important to determine whether HPV-11 and -18 E6 are ubiquitinated. To investigate this possibility, HT1080 cells were co-transfected with an expression construct for HA-tagged ubiquitin (Treier et al., 2004). To determine whether the ubiquitin-proteasome system was involved in the degradation of the HPV E6 proteins, we treated HT1080 cells with MG-132 to inhibit proteasome function. The protein half-life analysis was carried out as described above. As shown in Fig. 3(A), control GFP-expressing cells in the absence of the CHX showed an increase in fluorescence over time, while CHX-treated cells showed a minor reduction in fluorescence levels. Cells treated with CHX and MG-132 showed fluorescence levels similar to CHX alone. These results are consistent with GFP not being targeted for proteasome-mediated degradation, resulting in the relatively long half-life of GFP. In comparison to native GFP, fusing the HPV-11 and -18 E6 proteins to GFP markedly reduced fluorescence in a time-dependent manner, which was clearly most evident in the presence of CHX where the estimated half-lives were approximately 7 h for both GFP-11E6 and GFP-18E6 proteins (Fig. 3B, C). However, inhibition of the proteasome with MG-132 in the CHX-treated cells restored the half-lives of GFP-11E6 and GFP-18E6 to levels similar to the control cells, confirming that proteasome inhibition increased the half-lives of the GFP-E6 fusion proteins (Fig. 3B, C). Thus, proteasome inhibition significantly reduced the degradation rates of both GFP-11E6 and GFP-18E6, to the same extent.
Fig. 3. Protein half-life analysis of GFP-E6 fusion proteins determined by flow cytometry and Western blotting in the absence and presence of proteasome inhibitor. Cells were transfected with GFP, GFP-11E6 or GFP-18E6, split into 60 mm dishes after 9 h to equalize transfection efficiencies, and after 24 h cells were treated with either 100 μg CHX ml⁻¹ or 100 μg CHX ml⁻¹ with 50 μM MG-132 for 0, 2, 5 and 9 h. Control cells were incubated in equal volumes of solvent. Cells were collected, washed twice, and resuspended in PBS with 1% FBS. Green fluorescence of 10,000 cells was measured by FACSscan. The percentage of fluorescent cells was plotted for: (A) GFP; (B) GFP-11E6; and (C) GFP-18E6. An arbitrary fluorescence of 100% was attributed to cells collected at 0 h. Results represent the mean of three experiments, and error bars indicate standard error of the mean. (D) Western blot analysis of protein stabilities of GFP-E6, in the absence and presence of proteasome inhibitor. Cells were transfected as described above and, 24 h post-transfection, cell lysates were prepared by sonication at 0 and 5 h following treatment with either 100 μg CHX ml⁻¹ or 100 μg CHX ml⁻¹ with 50 μM MG-132. Control cells were incubated in equal volumes of solvent and Western blotting was performed using anti-GFP antibody. Lower panels show reprobing with anti-actin antibody, to ensure equal loading. (E, F, G) Bands representing GFP and GFP-E6 were quantified by peak density using a VersaDoc Imaging system and Quantity One software (Bio-Rad). An arbitrary density of 100% was attributed to the 0 h time-point. (E) GFP; (F) GFP-11E6; and (G) GFP-18E6.
1994) and expression plasmids for FLAG-11E6 and FLAG-18E6, using the vaccinia virus/T7 system as described previously (Li et al., 1999). Cells were harvested 24 h post-transfection and protein extracts were subjected to immunoprecipitation with anti-FLAG antibody and protein A–agarose (Pharmacia). Immunoprecipitated and non-immunoprecipitated lysate (W.C. 10% input) were then subjected to Western blot analysis using either anti-FLAG or anti-HA antibody (HRP-conjugated mouse monoclonal IgG; ICN). As shown in Fig. 4 (lane 3, upper panels A and B of the anti-HA Western blots), high molecular mass, poly-ubiquitinated E6 proteins were only detected in cells co-transfected with the HA-tagged ubiquitin vector and the FLAG-tagged E6 expressing constructs. This revealed that both FLAG-11E6 and FLAG-18E6 proteins were ubiquitinated.

Differences in the banding pattern of polyubiquitinated E6 proteins are apparent, where only the high molecular band of 200 kDa was detected for FLAG-18E6 and multiple polyubiquitinated bands were detected for FLAG-11E6.
(Fig. 4 A, B, upper panels, lane 3). This is, however, consistent with the anti-FLAG Western blots in the lower panels of Fig. 4, showing higher expression levels for FLAG-11E6 than for FLAG-18E6. The higher expression of FLAG-11E6, as compared to FLAG-18E6, using the vaccinia virus/T7 transfection system has been observed previously by Li et al. (1999). Due to the lower levels of FLAG-18E6 expression, detection of ubiquitinated protein becomes limited with the anti-FLAG antibody since there is only a single FLAG epitope on the ubiquitinated 18E6 proteins. Thus, detection of ubiquitinated FLAG-18E6 is not observed in lane 3 of the anti-FLAG Western blot in the lower panel of Fig. 4(A). However, since this polyubiquitinated species of FLAG-18E6 contained multiple HA epitopes due to the addition of numerous HA-ubiquitin molecules and only one FLAG epitope, the anti-HA Western blot was far more sensitive than the anti-FLAG Western blot. Therefore, the 200 kDa band, representing polyubiquitinated FLAG-18E6, was clearly detected in lane 3 of the upper panel of Fig. 4(A). The increased sensitivity of the anti-HA Western blot explains the increase in the number of polyubiquitinated FLAG-11E6 species detected, as compared to the anti-FLAG Western blots. Taken together, these data demonstrate that HPV-11 and -18 E6 are both susceptible to ubiquitination, thus supporting the previous experiments and arguing that the E6 proteins from low- and high-risk HPV types can be degraded via the ubiquitin-mediated proteasome degradation pathway.

A recent study reported that high-risk HPV-16 and -18 E6, but not the low-risk HPV-6a and -11 E6 proteins, were stabilized by inhibition of the proteasome degradation pathway (Kehmeier et al., 2002). Our results are in agreement with this study with respect to the high-risk HPV-18 E6 and we further extended these observations by showing that these E6 proteins become ubiquitinated. However, our observations differ with respect to the low-risk HPV types. In the present study, we showed that the HPV-11 E6 protein was stabilized by proteasome inhibition and this was supported by the observation that HPV-11 E6 was polyubiquitinated. Furthermore, fusing HPV-11 and -18 E6 to GFP reduced the half-life of GFP in the presence of CHX and this was reversed upon the addition of proteasome inhibitor.

Differences in the observations regarding the low-risk HPV-11 E6 may be due to the different experimental design in which Kehmeier et al. (2002) used a C-terminal AU epitope-tagged E6, whereas the present study used N-terminal tagged E6 proteins. Despite retaining the ability to target p53 for degradation, we observed that C-terminal AU-tagged E6 was unable to mediate the degradation of PDZ-containing MAGI-1. Since PDZ domain-containing proteins represent a major target for high-risk HPV E6 proteins, we used N-terminal tagged E6, which is functional for targeting MAGI-1, in this study.

It is, however, important to highlight that the present study supports the conclusion of Kehmeier et al. (2002) with respect to high-risk HPV-18 E6 being a target for proteasome degradation. Certainly, this is an important development in defining the regulation of this oncogenic protein in HPV-infected cells, and therefore merits independent confirmation as provided by this study.

In summary, the present study reveals a potentially important common trait, arguably the first identified biochemical process shared between low- and high-risk HPV E6 proteins, with respect to both being targets for ubiquitination and proteasome-mediated degradation. In addition to acting as a target for degradation, it is possible that ubiquitination may enhance biological function(s) of the E6 proteins. Recent reports have shown roles for ubiquitination in cellular trafficking, kinase activation and transcriptional regulation (reviewed by Pickart, 2001), and may be relevant with respect to HPV E6 modification by ubiquitin. However, since both low-risk and high-risk HPV E6 proteins became polyubiquitinated this argues that ubiquitination does not contribute to the oncogenic properties of high-risk HPV E6. Exploring the mechanisms involved in the ubiquitination and proteasome-mediated degradation of HPV E6 will shed light on the significance of these phenomena in the context of the virus life-cycle and the pathology of infection.

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