Online ISSN: 2682-2628
Print ISSN: 2682-261X

IJCBR

International Journal of Cancer and Biomedical Research

https://jcbr.journals.ekb.eg

Tax-interacting protein 1 (TIP-1) is a degradation target of HPV-16 E6 in human cervical cancer

Mona Elhelaly, Miranda Thomas, Paola Massimi and Lawrence Banks

PUBLISHED BY
EACR EGYPTIAN ASSOCIAN FOR CANCER RESEARCH
Since 2014

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

RESEARCH ARTICLE

Tax-interacting protein 1 (TIP-1) is a degradation target of HPV-16 E6 in human cervical cancer

Mona Elhelaly[1,2], Miranda Thomas[2], Paola Massimi[2] and Lawrence Banks[2]

[1]Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[2]Tumor Virology Group, I.C.G.E.B., AREA Science Park, Padriciano 99, Trieste, Italy

ABSTRACT

Background: The high-risk Human Papilloma virus E6 oncoprotein plays an essential role in the development and maintenance of malignancy through its ability to target several PDZ domain containing proteins one of them is a small PDZ protein called Tax-interacting protein 1 (TIP-1). Tip-1 is known to be implicated in carcinogenesis through regulation of Rho and wnt/β-catenin signaling pathways. It also regulates many cellular processes through its interaction with several cellular proteins, including the tax protein, LIN7, MAGI-1, PlexinD1, AHDC1, L-glutaminase and Kir2.3. **Methods:** GST pull down assays, In vitro degradation assays, cell culture and co-transfection, Immunofluorescence assays and Western blotting were used to analyze cellular expression levels of TIP-1. **Result:** TIP-1 was shown to be degraded when co-transfected with E6s from three different cancer-causing HPV types (HPV-16, HPV-18, and HPV-33E6) co-transfection was repeated in the presence of CBZ and we show by western blot the appearance of Tip-1 again. Also, the co-transfection was repeated in the absence of E6AP and the appearance of Tip-1 again was shown by western blot. Immunofluorescence assay have shown an increase of Tip-1expression in presence of CBZ. **Conclusion:** ITip-1 is degraded by three different cancer-causing HPV types (HPV-16, HPV-18, and HPV-33E6) This degradation also appears to be dependent on the presence of the E6-AP (UBE3A) protein-ubiquitin ligase. This suggests that TIP-1 is a common target of high-risk HPV types.

Keywords: E6-AP, HPVE6, Human cancer cervix, TIP-1

Editor-in-Chief: Prof. M.L. Salem, PhD - Article DOI: 10.21608/JCBR.2023.186125.1289

ARTICLE INFO

Article history
Received: January 8, 2023
Revised: May 1, 2023
Accepted: May 29, 2023

Correspondence to
Mona Elhelaly,
Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt
Tel.: 01149499759
Email: dr_mona_elhelali@mans.edu.eg
ORCID: https://orcid.org/0000-0001-8612-4247

INTRODUCTION

Infection with human papillomaviruses (HPVs) causes around 99% of all cervical carcinomas, an estimated number of more than 600,000 cancer cases annually worldwide (Bansal et al., 2016). HPV types that are associated with human malignancies are referred to as high-risk (HR) types, with HPV-16 and 18 being the most common HR HPV types found in cervical carcinoma (zur Hausen, 2009). The E6 oncoproteins of high-risk HPVs play an essential role in the development and maintenance of malignancy, in part through their ability to target several PDZ domain-containing proteins. These include the tumor suppressor p53, the pro-apoptotic protein Bak, and a number of PDZ (PSD95/Dlg/ZO) domain-containing cell polarity regulators, including Dlg, Scribble, and MAGI-1(Banks et al., 2012).

Many of the HR E6 oncoprotein's functions are mediated through its ability to recruit a cellular ubiquitin ligase, E6AP, which plays an important role in the ubiquitin-directed, proteasome-mediated degradation of many E6 substrates (Huibregtse et al., 1993).

One of E6's PDZ domain-containing targets is the Tax-interacting protein 1 (TIP-1), also known as Tax1BP3 or GIP, which has been reported to be a target of HPV-16 E6 (Hampson et al., 2004). TIP-1 is a small protein of 124 amino acids, highly conserved between humans and mice (Han et al., 2012). The structural and functional unit of TIP-1 is a single PDZ domain (of 89 amino acids); thus, differentiating TIP-1 from other PDZ domain-containing proteins, which usually contain multiple structural and functional domains and can serve as scaffolds in assembling large protein complexes (Yen et al., 2016).

TIP-1 plays an important role in many biological events through its binding to a growing list of interacting protein partners (Mohanty et al., 2015). TIP-1 is involved in Rho signaling through interaction with rhotekin (Reynaud et al.,2000), it also interacts with the potassium ion channel Kir 2.3 and regulates cell polarity (Alewine et al., 2006), furthermore TIP-1 was identified as a target of two different human viruses: human T-lymphotropic virus type I (HTLV-1)(Wang et al., 2014) and human papillomavirus (HPV). TIP-1 is involved in regulation of Wnt signaling pathways through the transcriptional regulation of β-catenin (Kanamori et al., 2003), which it binds with very high affinity (Zhang et al., 2008).The oncogenic potential of TIP-1 has been identified in many studies, thus suggesting the potential of TIP-1 as a prognostic biomarker and therapeutic target; it is over-expressed in glioblastoma, lung, head-and-neck, and breast cancers and is associated with alterations in cell adhesion, migration, and metastasis (Han et al., 2012; Wang et al., 2014). Tip-1is also a radiation-inducible neo-antigen which plays an important role in tumor control (Yan et al., 2016).

It has been demonstrated that TIP-1 inhibits colon cancer cell proliferation, suggesting that TIP-1 may function as a tumour suppressor. However, higher TIP-1 expression levels were discovered in human invasive breast cancer and human glioblastoma, and their correlation with advanced stages and a bad prognosis raised the possibility that TIP-1 may have an oncogenic function. In this study, we examined the interaction of TIP-1 with the E6 oncoproteins from a number of high-risk HPV types. We show that exogenous TIP-1 is degraded in a proteosome-dependent manner in the presence of the E6 oncoproteins from high-risk HPV types -16, -18 and, -33. This degradation also appears to be dependent on the presence of the E6-AP (UBE3A) protein-ubiquitin ligase. This suggests that TIP-1 may be a common target of high-risk HPV types.

MATERIALS AND METHODS

All experiments were performed at least thrice.

Cell Culture and Transfection

HEK293, HaCat, HeLa, CaSki, and SiHa cells were purchased from the American Type Culture Collection (Manassas, VA, USA) and maintained in Dulbecco's modified Eagles Medium (DMEM), supplemented with 10% fetal bovine serum and penicillin-streptomycin (100 U/ml) in a 37°C humidified incubator containing 10% CO2.

Degradation assays *in vivo*

HEK293 cells were transfected with pCDNA3: FLAG-TIP-1 alone or together with either pCDNA3: HA-16E6, pCDNA3: HA-18E6, or pCDNA3: HA-33E6. A pCDNA3 empty vector was used as a negative control and to balance the DNA inputs. A plasmid expressing β-galactosidase was included as a transfection control in each sample. After 18h, cells were harvested into SDS-PAGE sample buffer and analysed by SDS–PAGE and Western blotting, as described previously (Massimi et al., 2004). The blots were probed with mouse anti–FLAG monoclonal antibody (Sigma), with HRP-conjugated anti-mouse, and visualised using the ECL system (Amersham), according to the manufacturer's instructions. Blots were re-probed for β-galactosidase to control for transfection efficiency. The cotransfection was repeated in the presence and absence of CBZ proteasome inhibitor, and in the presence and absence of E6AP.

Expression of GST fusion proteins

HPV-16, -18 and -33 E6 genes were cloned into pGEX.2 and transfected into DH5α (rec-) cells. 50 ml aliquots were grown at 37°C overnight in Luria broth (LB) and then passage into 500ml of LB. After growth for 1 h at 37°C, GST-fusion protein expression was induced by addition of IPTG (isopropylthiogalactopyranoside), to a final concentration of 1mM. After incubation for 3h at 37°C, the cells were pelleted and resuspended in 5ml PBS, 0.5% Triton X-100, then sonicated on ice for 30s. The cell debris was removed by centrifugation and the supernatant incubated at 4°C overnight with glutathione-conjugated agarose (Sigma) on a rotating wheel.

GST fusion protein binding assays

The fusion proteins conjugated to Glutathione agarose were washed three times with PBS alone. A 10µl sample was analysed for protein concentration by SDS-PAGE and stained with Coomassie Brilliant Blue. The in vitro translated

TIP-1 was then incubated with aliquots of the agarose-conjugated GST fusion proteins, equalised for fusion protein concentration. Incubations were performed on ice with shaking; after 1h the samples were washed with PBS, 0.1% Nonidet-P40, and the bound proteins were analysed by SDS-PAGE and autoradiography.

***In vitro* degradation assays**

Proteins were transcribed and translated in vitro in rabbit reticulocyte lysate using the Promega TNT system according to the manufacturer's instructions. The HPV-16, HPV-18, and HPV-33 E6 proteins were radiolabelled with [35S]-cysteine, while TIP-1 was radiolabelled with [35S]-methionine. Degradation assays were performed as previously described (Thomas et al., 2001).

siRNA

For all siRNA transfections, the cells were seeded on 6cm dishes at 1.2×10^5 cells per dish. The following siRNAs were purchased from Dharmacon: si-Luciferase, si-HPV18 E6/E7, si-E6AP and si-TIP-1 and were transfected using RNAiMAX reagent (Invitrogen) according to the manufacturer's instructions.

Western immunoblotting and Antibodies

The following antibodies were used:

- Mouse monoclonal anti-FLAG (M2), Mouse moncoclonal anti-HA, Mouse monoclonal anti-β-galactosidase, Rabbit polyclonal anti-TIP-1, and mouse monoclonal anti-α-tubulin were purchased from Sigma Aldrich. Mouse monoclonal anti-p53 (DO-1), mouse monoclonal anti-α-actinin, and Mouse anti-HPV E6 were purchased from Santa Cruz Biotechnology.
- Appropriate HRP-
- coupled secondary antibodies were purchased from DAKO.
- Short exposure for 2 second was enough for the detection of TIP-1 While long exposure for 10 second was done to detect tip-1 isoform.

Immunofluorescence

HPV-18-positive HeLa and HPV-16-positive CaSki cells were seeded lysine coated glass coverslips in a six well plate and grown overnight. They were treated with or without CBZ for 4h After this, cells were washed twice with phosphate-buffered saline (PBS) and fixed for 20 min with 4% paraformaldehyde at room temperature, and then washed three times with PBS. The cells were permeabilized with PBS containing 0.1% Triton X-100 for 5 min at room temperature. After permeabilization, cells were washed three times with PBS then incubated with rabbit anti TIP-1 antibody (1:100 dilution) for 2 h at 37°C in a humidified chamber. Cells were then washed for 5 min with PBS three times and then incubated with secondary antibody (1:600 dilution) for 30 min at 37°C in a humidified chamber. The secondary antibodies used were fluorescein isothiocyanate (FITC) and rhodamine conjugated (Invitrogen). followed by three 5-min washes with PBS. The images were obtained with a Leica DMLB fluorescence microscope

Transformation suppression assays

HPV-negative HaCat cells and HPV-positive HeLa, CaSki and SiHa cells were seeded onto 6cm dishes at $1.2x10^5$ cells per dish or in 6-well plates at $3x10^4$ cells per well and grown overnight. They were then transfected with pCDNA3.1: TIP-1, or with empty vector using Lipofectamine (Invitrogen) and incubated for 24h. Fresh medium containing xmg ml neomycin (G418) was then added and the cells were grown for 3 weeks, changing the medium every two days. After overnight incubation with Giemsa stain the colonies were counted. Each condition was performed in duplicate in 5 separate experiments.

RESULT

High-risk HPV E6 proteins induce degradation of TIP-1 in vivo.

The E6 oncoproteins of high-risk HPV types interact with a large number of cellular proteins, and this interaction often leads to the proteasomal degradation of many of these E6 target proteins. Although TIP-1 has long been known to be a target of the HPV-16 E6 oncoprotein (Hampson et al., 2004), it was not clear whether E6 could induce its degradation. To investigate this, HEK-293 cells were transfected with a plasmid expressing Flag-tagged TIP-1, either alone or with plasmids expressing HA-tagged HPV-16 E6, HPV-18 E6 or

HPV-33 E6, in duplicate. After overnight incubation, one set of the cells was treated with the proteasome inhibitor CBZ for 2hours prior to protein extraction. The total cell extracts were separated by SDS-PAGE and Flag-TIP-1 levels were analysed by immunoblotting. The results in Figure 1A show that Flag-TIP-1 protein is completely degraded in the presence of each of the E6 oncoproteins, but that TIP-1 levels are restored in the presence of a protease inhibitor. This indicates that the high-risk E6 proteins are capable of inducing TIP-1 degradation in a protease-dependent manner.

HPV E6 induces the degradation of a number of its target proteins, including p53, Bak, and MAGI-1 (Huibregtse et al 1991, Thomas and Banks 1998, Glaunsinger et al 2000) through redirecting the ubiquitin-protein ligase E6AP (E6-associated protein, or UBE3A). To examine whether E6-induced degradation of TIP-1 is mediated by E6AP, HEK-293 cells were again transfected with a plasmid expressing Flag-tagged TIP-1, either alone or with plasmids expressing HPV-16 E6, HPV-18 E6, or HPV-33 E6, in duplicate. After overnight incubation, one set of cells were treated with siRNA to Luciferase and the other with siRNA to E6AP. After 24h, the cells were harvested, separated by SDS-PAGE and Flag-TIP-1 levels were analyzed by immunoblotting. The results in Figure 1B show that, in the absence of E6AP, E6-induced degradation of TIP-1 was prevented, indicating that E6-induced TIP-1 degradation can be mediated through E6AP.

Having shown that high-risk E6 could induce the degradation of exogenously expressed TIP-1, it was of interest to know what the status of TIP-1 was in cells derived from HPV-induced cancers endogenously expressing E6. To examine this, we treated HPV-16-positive CaSki and SiHa cells, and HPV-18-positive HeLa cells, with the CBZ proteasome inhibitor. After treatment, the cells were harvested and the levels of TIP-1 protein were examined by Western Blot, as before.

In Figure 2A, it can be seen that the three cell lines show interesting differences in their TIP-1 levels: CaSki and HeLa cells show a slight increase in TIP-1 levels upon inhibition of the proteasome, while TIP-1 levels in SiHa cells do not appear to be affected by CBZ treatment.

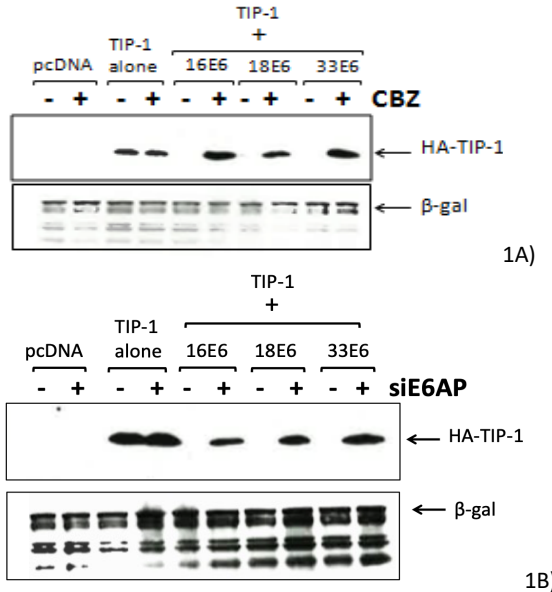
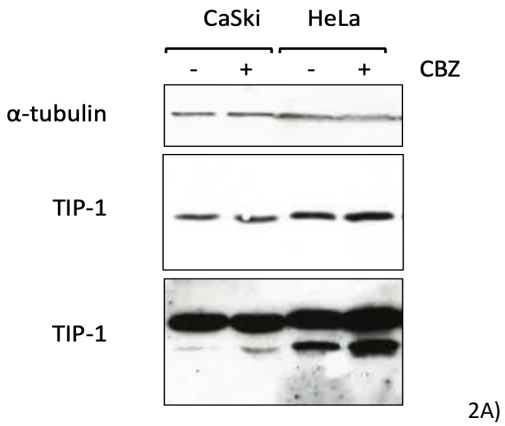
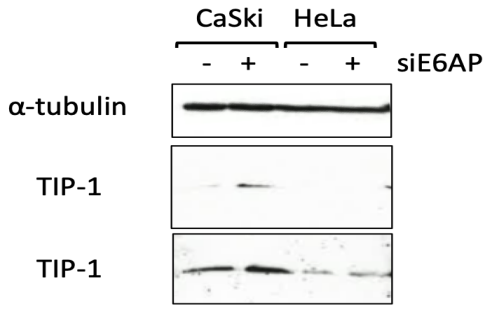


Figure 1. High-risk HPV E6 proteins induce degradation of HA-TIP-1 in HEK293 cells in a proteasome and E6-AP-dependent manner. **A**. Analysis of exogenous HA-tagged TIP-1 levels using a Western blot in the presence or absence of exogenous E6 proteins from high-risk HPV-16, -18, and -33, as well as the proteasome inhibitor CBZ. Anti-HA antibody was probed on the upper panel. As a transfection effectiveness check, a plasmid expressing -galactosidase was co-transfected (lower panel). **B.** HA-TIP-1 plasmids were transfected into HEK293 cells as before, either with or without plasmids expressing high-risk E6 proteins. Cells were co-transfected with siRNA to E6AP or luciferase after 24 hours. Cells were harvested and analysed by Western blot after 48 hours. Anti-HA antibody was probed on the upper panel. As a transfection effectiveness check, a plasmid expressing -galactosidase was co-transfected (lower panel).

Interestingly, in HeLa cells, the level of TIP-1 alternatively spliced protein also appears to increase in response to proteasome inhibition. To examine the involvement of E6AP, CaSki, SiHa, and HeLa cells were transfected with either siRNA to E6AP or a scrambled siRNA. After 48h the cells were harvested and the TIP-1 levels analyzed by Western blot as before. As can be seen in Figure 2B, TIP-1 levels in CaSki cells are partially rescued by suppressing E6AP expression, while in HeLa and SiHa cells the levels do not appear to change, suggesting that E6AP is not essential in the control of TIP-1 levels in these cells.



2A)



2B)

Figure 2. Endogenous TIP-1 in CaSki and HeLa cells is targeted for degradation. **A.** CaSki and HeLa cells were treated for 3h with proteasome inhibitor CBZ prior to harvest and analysis by Western Blot. The upper panel shows the α-tubulin loading control. The lower panels show that endogenous TIP-1 levels increase slightly upon proteasome inhibition, particularly the shorter alternatively spliced form of TIP-1 **B.** Endogenous TIP-1 levels also increase upon siRNA knockdown of E6AP.

TIP-1 localisation in cervical cancer-derived cell lines

Having shown that the HPV-transformed cell lines differ in their induction of TIP-1 degradation, we were interested in knowing whether they affected intracellular localization. To examine this, we plated CaSki and HeLa cells on glass coverslips. After overnight growth, the cells were treated with either DMSO alone or CBZ proteasome inhibitor for 3 hours; after fixation, the cells were examined by immunofluorescence and probed with anti-TIP-1 antibody.

As can be seen from Figure 3, TIP-1 (green) is cytoplasmic in untreated CaSki cells and is more diffuse in HeLa cells. However, in both cell lines, upon proteasome inhibition, shown by the increase in p53 levels (red), the levels of nuclear TIP-1 rise markedly. This suggests that E6 primarily targets nuclear TIP-1.

Effect of TIP-1 overexpression on the growth of HPV-positive cervical cancer-derived cell lines

Having shown that HPV E6 can induce the degradation of TIP-1, we asked whether TIP-1 expression might have a negative effect on cell growth. To examine this, we performed a transformation suppression assay. HPV-negative HaCat cells and HPV-positive HeLa, CaSki, and SiHa cells were transfected with pCDNA3.1: TIP-1, or with empty vector. After 24 h, the cells were put under G418 selection and grown under selection for 3 weeks. Cells were then fixed and stained overnight with Giemsa stain. The numbers of colonies were counted and the results are shown in Figure 4B, where the colony numbers in the presence of exogenous TIP-1 are expressed as a percentage of the colony numbers in cells transfected with empty vector. The histogram represents the collated results of 5 assays. It is clear that the expression of TIP-1 has a tumour suppressive effect in HPV-16 expressing CaSki and SiHa cells but has little or no effect upon the growth of HPV-negative cells or HPV-18 expressing HeLa cells.

DISCUSSION

High-risk HPV types are distinguished by having a PDZ-binding motif (PBM) at the extreme C-terminus of the E6 oncoprotein. Through this motif, E6 targets a number of PDZ domain-containing proteins, mainly for proteasome-mediated degradation (Thomas et al., 2016; Ganti et al., 2015; Allouch et al., 2019). TIP-1, reported to be an E6 target (Hampson et al., 2004; Oliver et al., 2011), is a unique PDZ protein in that it consists almost solely of the PDZ binding pocket (Dadey, 2019), and it has thus been speculated that TIP-1 might prevent certain PBM-PDZ interactions by binding the PBM itself and blocking the interaction.

In this study, we examined the interaction of TIP-1 with high-risk HPV E6 proteins. We find that, although we were unable to show binding between in vitro-translated TIP-1 and GST-E6 proteins (data not shown), the levels of exogenously expressed TIP-1 are reduced in the presence of high-risk HPV-16, -18 and, -33 E6 proteins in a proteasome- and E6-AP-dependent manner.

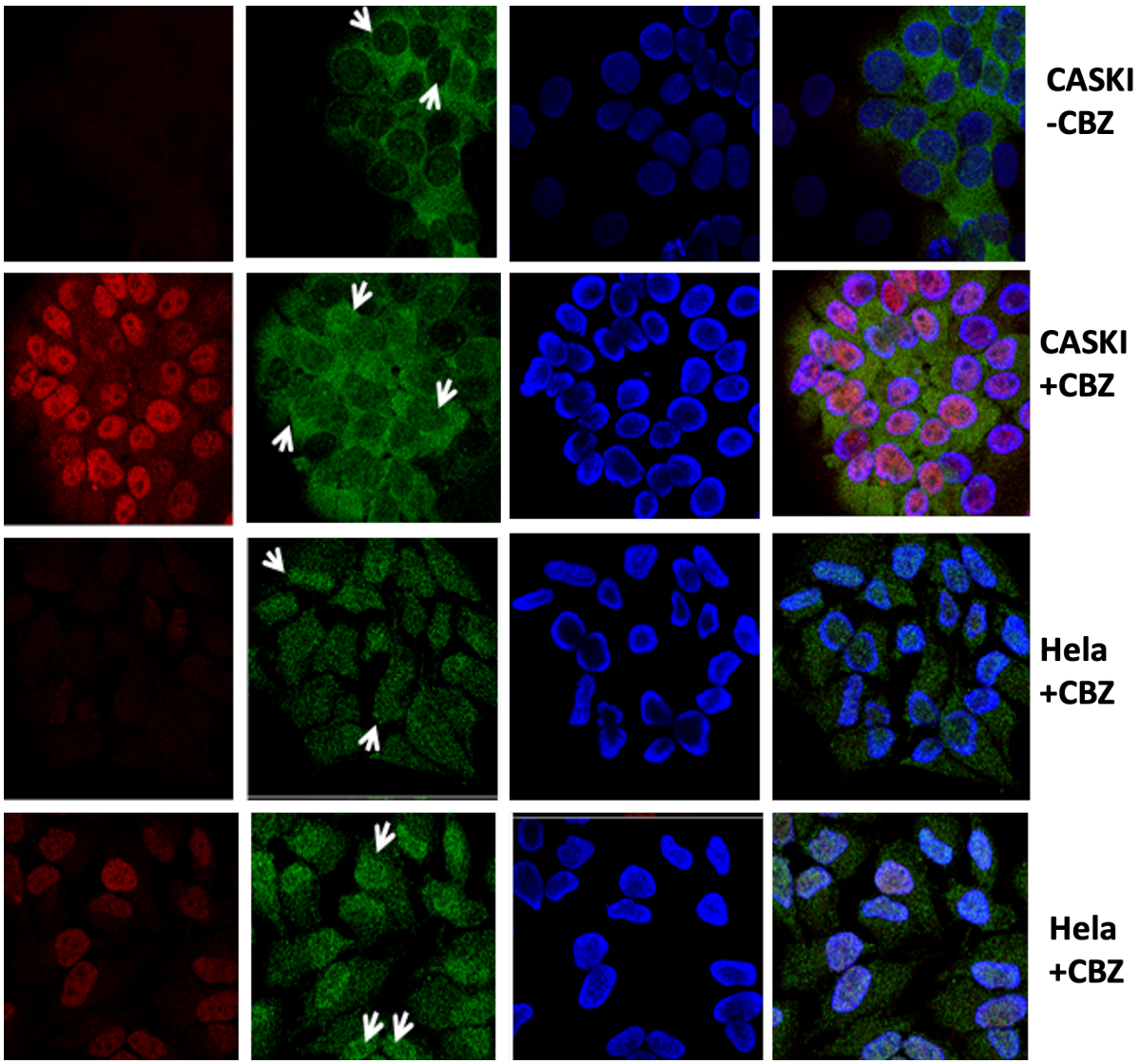


Figure 3. HPV-16 and HPV-18 E6 proteins target a nuclear pool of TIP-1 for degradation. CaSki and HeLa cells were seeded on coverslips overnight. After 3h incubation with CBZ proteasome inhibitor the cells were fixed and subjected to Immunofluorescence analysis, using anti-TIP-1 antibody (green), anti-p53 antibody (red) to demonstrate proteasome block, and DAPI (blue) to show nuclei.

There could be a number of reasons for not detecting binding in vitro, the simplest of which is that the interaction is probably not between monomeric E6 or TIP-1 molecules, but involves a dimer of multimer of one or both, and this is supported by reports that TIP-1 forms dimers in solution (Aledo et al., 2001). The finding that TIP-1 levels are decreased in the presence of high-risk HPV E6 proteins was in some ways surprising, since a previous report (Hampton et al., 2004) did not note any degradation. In the assays reported here, the ratio of E6-expressing plasmid to TIP-1-expressing plasmid was 3:1, and at this level of E6 expression the reduction in TIP-1 protein levels is clear. At transfection ratios of 1:1, this difference is reduced, suggesting that TIP-1 degradation may need higher concentrations of E6, which, in turn, may explain why the proteasomal degradation of TIP-1 in the presence of E6 was not previously detected. Having shown that exogenous TIP-1 is subject to degradation in the presence of E6, we wished to determine whether endogenous TIP-1 is also degraded and, if so, whether the degradation is dependent on E6AP. In HPV-16-positive CaSki cells and HPV-18-positive HeLa cells, treatment with a proteasome inhibitor increases TIP-1 levels, as does si-RNA-mediated knockdown of E6AP, supporting the results obtained in the transient expression system.

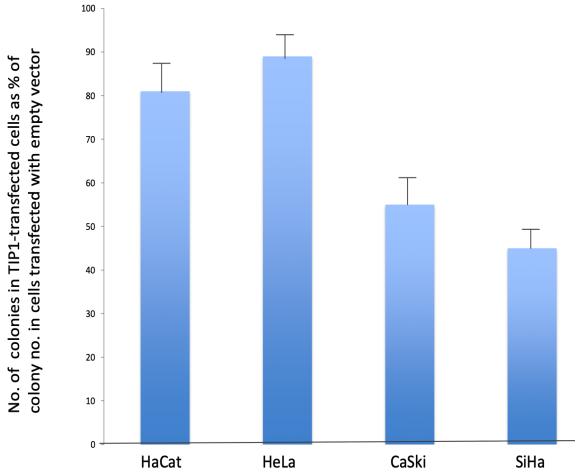


Figure 4. TIP-1 can suppress transformation in HPV-16-positive cancer cells. Transformation suppression assays were performed in HPV-negative HaCaT cells, HPV-18-positive HeLa cells and HPV-16-positive CaSki and SiHa cells. Cells were transfected with plasmid expressing TIP-1 and held under G418 selection for 3 weeks. The cells were then fixed and stained with Giemsa and colonies counted. The histogram shows the collated results of five assays, expressed as number of colonies as a percentage of cells transfected with empty vector. Bars showed mean +/- SD.

However, it is important to note that the effect is not strong and that TIP-1 levels are easily detectable in the absence of the proteasome inhibitor, which may suggest that a specific cellular pool of TIP-1 is the target of E6-induced degradation. In support of this supposition, it is noted in Figure 2A that the lower molecular weight isoform of TIP-1 appears to be preferentially targeted for degradation.

To examine the possibility that a specific cellular pool of TIP-1 is the target of E6, we performed immunofluorescence assays on Caski and HeLa cells with and without proteasome inhibition. It is first interesting to note that in the absence of inhibition, the cells appear quite different, with TIP-1 being almost exclusively cytoplasmic in Caski cells, while HeLa cells show a low level of diffuse staining throughout. Upon proteasome inhibition, visualised by staining for p53, the levels of TIP-1 increase in both cell lines and most notably in the nucleus, suggesting that high-risk HPV E6 primarily targets a nuclear pool of TIP-1 for degradation. It is known that TIP-1 can antagonise the transcriptional activation function of β-catenin (Kanamori et al., 2003; Zhang et al., 2008) which is obviously a nuclear activity, and it is possible that high risk HPVs target nuclear TIP-1 to relieve that transcriptional repression and allow increased cell proliferation. It is interesting that the cytoplasmic TIP-1 does not appear to be as strongly targeted, if at all, by E6, which would leave it free to target the pool of β-catenin that has a role in the maintenance of cell junctions and epithelial integrity (Brembeck et al., 2006). In this context, it is notable that TIP-1 has been shown to have proto-oncogenic potential in facilitating cell migration and metastasis in breast cancer cells (Han et al., 2012).

With that in mind, we examined the effect of re-expressing TIP-1 exogenously in HPV-positive cervical cancer cell lines and found that the growth of HPV-16-positive CaSki and SiHa cells was markedly inhibited by TIP-1 overexpression, while no effect was seen in HPV-negative HaCaT or HPV-18 positive HeLa cells. This correlates with other studies showing that HPV-16 has a stronger effect upon TIP-1 than HPV-18 and binds it with a higher affinity (Hampson et al., 2004; Oliver et al., 2006; Thomas et al., 2016). This may suggest that, during the process of transformation, HPV-16 and HPV-18 transformed cells differ in their dependence on TIP-1 degradation as a means of maintaining their growth advantage. HPV-16 and HPV-18 E6 proteins have previously been shown to have quite marked differences in their substrate selection, and this may well be another example of this difference (Thomas et al., 2005; 2016).

CONCLUSION

In conclusion, Tip-1 is degraded by three different cancer-causing HPV types (HPV-16, HPV-18, and HPV-33E6) This degradation also appears to be dependent on the presence of the E6-AP (UBE3A) protein-ubiquitin ligase. This suggests that TIP-1 is a common target of high-risk HPV types.

LIMITATION

Our study has some limitations, as the study focused only on high-risk HPV E6 oncoprotein and limited to certain cell lines this might limit the generalizability of the result however we focused on cancer cervix cell lines which may considered as a point of strength.

OUTLOOK

Extend our research to investigate the relation between TIP-1 and other high-risk HPV

oncoproteins e.g HPV E7. Investigate different cancer pathways to detect exactly how TIP-1 gene may affect carcinogenesis.

ACKNOWLEDGEMENTS

M.E. was the recipient of an ICGEB Short Term Research Fellowship. L.B. gratefully acknowledges support from the Associazione Italiana per la Ricerca sul Cancro.

CONFLICT OF INTEREST

All authors declare no conflicts of interest.

FUNDING

No fund was received for this work.

REFERENCES

Aledo JC, Rosado A, Olalla L, Campos JA, Marquez J (2001). Overexpression, purification, and characterization of glutaminase-interacting protein, a PDZ-domain protein from human brain. Protein Expr. Purif. 23(3): 411-418.

Alewine C, Olsen O, Wade JB, Welling PA (2006). TIP-1 has PDZ scaffold antagonist activity. Mol.Biol. Cell 17(10): 4200-4211.

Allouch, S., Malki, A., Allouch, A., Gupta, I., Vranic, S., & Al Moustafa, A. E. (2020). High-risk HPV oncoproteins and PD-1/PD-L1 interplay in human cervical cancer: recent evidence and future directions. *Frontiers in Oncology*, *10*, 914.

Banks L, Pim D, Thomas M (2012). Human tumour viruses and the deregulation of cell polarity in cancer. Nat. Rev. Cancer 12(12): 877-886

Bansal A, Singh MP, Rai B (2016) Human papillomavirus-associated cancers: A growing global problem. Int. J. Apl.Basic Med.Res. 6(2): 84-89

Brembeck FH, Rosário M, Birchmeier W (2006). Balancing cell adhesion and Wnt signaling, the key role of beta-catenin. Curr. Opinion Genetics & Dev. 16 (1): 51–9.

Dadey, D. Y. A. (2019). Targeting Tax Interacting Protein 1 Results in Cytotoxic and Radiosensitizing Effects in Cancer. *Neurosurgery*, *66*, 310-316.

Ganti K Broniarczyk J, Manoubi W, Massimi P, Mittal S, Pim D, Szalmas A, Thatte J, Thomas M, Tomaic V, Banks L (2015). The Human Papillomavirus E6 PDZ Binding Motif: From Life Cycle to Malignancy Viruses 7(7): 3530-3551.

Glaunsinger BA, Lee SS, Thomas M, Banks L, Javier R (2000). Interactions of the PDZ-protein MAGI-1 with adenovirus E4-ORF1 and high-risk papillomavirus E6 oncoproteins. Oncogene 19(46): 5270-5280.

Hampson L, Li C, Oliver AW, Kitchener HC, Hampson IN (2004). The PDZ protein Tip-1 is a gain of function target of the HPV16 E6 oncoprotein.Int J Oncol 25(5): 1249-1256.

Han M, Wang H, Zhang H-T, Han Z-Z (2012). Expression of TIP-1 confers radioresistance of malignant glioma cells. Biochem. Biophys Res. Comm.422: 139-145

Huibregtse JM, Scheffner M, Howley PM (1991). A cellular protein mediates association of p53 with the E6 oncoprotein of human papillomavirus types 16 or 18. EMBO J 10(13): 4129-4135.

Huibregtse JM, Scheffner M, Howley PM (1993). Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. Mol.Cell Biol.13(2): 775-784

Kanamori M, Sandy P, Marzinotto S, Benetti R, Kai C, Hayashizaki Y, Schneider C, Suzuki H. (2003) The PDZ protein tax-interacting protein-1 inhibits beta-catenin transcriptional activity and growth of colorectal cancer cells. J Biol. Chem. 278(40):38758-38764.

Massimi P, Gammoh N, Thomas M, Banks L (2004). HPV E6 specifically targets different cellular pools of its PDZ domain-containing tumour suppressor substrates for proteasome-mediated degradation. Oncogene. 2005 Sep 15;24(41):6222-30.

Mohanty S, Ovee M, Banerjee M (2015). PDZ Domain Recognition: Insight from Human Tax-Interacting Protein 1 (TIP-1) Interaction with Target Proteins. Biology (Basel) 4(1): 88-103.

Oliver AW He X, Borthwick K, Donne AJ, Hampson L, Hampson IN (2011). The HPV16 E6 binding protein Tip-1 interacts with ARHGEF16, which activates Cdc42. Br J Cancer 104(2): 324-331

Reynaud C, Fabre S, Jalinot P (2000). The PDZ protein TIP-1 interacts with the Rho effector rhotekin and is involved in Rho signaling to the serum response element. J Biol. Chem. 275 (43): 33962-33968.

Thomas M, Banks L (1998). Inhibition of Bak-induced apoptosis by HPV-18 E6. Oncogene 17(23): 2943-2954.

Thomas M, Glaunsinger B, Pim D, Javier R, Banks L (2001). HPV E6 and MAGUK protein interactions: determination of the molecular basis for specific protein recognition and degradation. Oncogene 20(39): 5431-5439.

Thomas M, Massimi P, Navarro C, Borg J-P, Banks L(2005). The hScrib/Dlg apico-basal control complex is differentially targeted by HPV-16

and HPV-18 E6 proteins. Oncogene 24(41): 6222-6230.

Thomas M, Myers MP, Massimi P, Guarnaccia C, Banks L (2016). Analysis of Multiple HPV E6 PDZ Interactions Defines Type-Specific PDZ Fingerprints That Predict Oncogenic Potential. PLoS Path. 12(8): e1005766

Wang H, Han M, Whetsell W, Wang J, Hallahan D, Han Z (2014). Tax-interacting protein 1 coordinates the spatiotemporal activation of Rho GTPases and regulates the infiltrative growth of human glioblastoma Oncogene 33(12): 1558-1569.

Yan H, Kapoor V, Nguyen K, Akers WJ, Li H, Scott J, Laforest R, Rogers B, Thotala D, Hallahan D. (2016). Anti-tax interacting protein-1 (TIP-1) monoclonal antibody targets human cancers. Oncotarget: 7(28):43352-43362.

Zhang J, Yan X, Shi C, Yang X, Guo Y, Tian C, Long J, Shen Y (2008). Structural basis of beta-catenin recognition by Tax-interacting protein-1. J. Mol. Biol. 384(1): 255-263

zur Hausen H (2009). Papillomaviruses in the causation of human cancers - a brief historical account.Virology. 2009 Feb 20;384(2):260-5.