Human Papillomavirus Oncoproteins and Ubiquitin Proteasome System

Subjects: Virology

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Papillomaviridae is a diverse family of small, non-enveloped DNA viruses, approximately 50–60 nm in diameter that infect all homoeothermic vertebrates including humans. Human papillomavirus (HPV) E6 and E7 oncoproteins are critical for development and maintenance of the malignant phenotype in HPV-induced cancers. These two viral oncoproteins interfere with a plethora of cellular pathways, including the regulation of cell cycle and the control of apoptosis, which are critical in maintaining normal cellular functions. E6 and E7 bind directly with certain components of the Ubiquitin Proteasome System (UPS), enabling them to manipulate a number of important cellular pathways.

Keywords: E6 ; E7 ; HPV ; cervical cancer ; proteasome ; UPS ; ubiquitin ligases ; ubiquitin

1. Introduction

Papillomaviridae is a diverse family of small, non-enveloped DNA viruses, approximately 50–60 nm in diameter that infect all homoeothermic vertebrates including humans [1][2]. Interestingly, recent studies have also detected members of *Papillomaviridae* in fish [3]. Currently, there are known to be approximately 200 different human papillomavirus (HPV) types, which are classified in five genera (alpha, beta, gamma, mu and nu) [4]. The *Alphapapillomavirus* species (α-HPVs) preferentially infect oral or anogenital mucosa in humans and primates [2]. The α-HPVs are further classified as low-risk (LR) (e.g., HPV-6 and HPV-11) or high-risk (HR) (e.g., HPV-16, HPV-18, and HPV-33), based on their association with human cancers [1]. HPV infections are usually fairly rapidly cleared by the immune system–from a few months up to two years from initial viral entry.

It is believed that HPVs infect cells in the basal layer of stratified squamous epithelia, exposed as a result of small abrasions and micro-wounds [5][6]. In normal epithelium, epithelial cells in the basal layer are proliferative, while the differentiating cells in the suprabasal layers have exited the cell cycle. The HPV life cycle is dependent upon the replicative machinery of basal cells, which have the potential to proliferate. Following infection, HPV genomes are established as extrachromosomal elements or episomes. Transition to the late stage of HPV infection is followed by the expression of late genes and virion production [5][7].

2. The Ubiquitin Proteasome System

The Ubiquitin Proteasome System (UPS) is the pathway responsible for the majority of intracellular protein degradation in eukaryotic cells. It controls the turnover of thousands of short-lived, regulatory, damaged and misfolded proteins, in order to regulate and maintain various cellular functions and cellular homeostasis [8]. It is a highly specific process and involves the addition of ubiquitin molecules to cellular substrates, which consequently leads to their modification via various cellular pathways. Fundamental cellular signaling pathways that are regulated by ubiquitination include cell cycle control, cell survival, cell proliferation, transcription, DNA repair, apoptosis, cellular metabolism, protein quality control, and membrane trafficking, as well as ubiquitination being very important in the immune response [9][10][11]. Ubiquitination, which involves a complex interplay of ubiquitinating and deubiquitinating enzymes (DUBs), is an essential element of control by the UPS [12][13]. Ubiquitin is transferred to target proteins by the ubiquitination cascade: first, ubiquitin is activated and bound by an E1 ubiquitin-activating enzyme, then transferred to an E2 ubiquitin-conjugating enzyme and, finally, transferred to lysine residues on the target protein by E3 ubiquitin-protein ligase [12][13]. So far, studies have identified two E1 activating enzymes, 40 E2 conjugating enzymes, around 800 E3 ubiquitin-protein ligases and about 100 DUBs [14]. While all components of the UPS are of great importance for ubiquitination, individual ubiquitin-protein ligases may be valuable viral targets as they are involved in substrate recognition, and hence define the specificity of the system. They are categorized into two main families: the numerous Really Interesting New Gene (RING) finger E3 ubiquitin ligases; and the less common Homologous to E6-associated protein C-Terminus (HECT) domain E3 ligases [15][16]. Polyubiquitin chains

attached to substrates via lysine 48 will result in target protein degradation at the proteasome, while polyubiquitin chain attachment to other lysines will modulate proteasome-independent activities [17]. Proteins can also be mono-ubiquitinated, and this influences processes such as endocytosis and vesicular sorting [18]. On the other hand, ubiquitin molecules are cleaved from substrates by DUBs [19], which play essential roles in cellular processes as diverse as DNA replication, gene silencing, and endocytosis, ultimately affecting cell growth and oncogenesis.

3. HPV and the UPS

Many human pathologies, such as inflammatory and neurodegenerative diseases and certain cancers are, directly or indirectly, promoted by the deregulation of the UPS [8][20]. An intriguing feature of both HPV oncoproteins, E6 and E7, is their ability to direct many of their cellular substrates for proteasome-mediated degradation [6][21]. Those interactions were either shown or confirmed using methods like co-immunoprecipitation, Glutathione-S-transferase (GST)-fusion protein pull-down, affinity chromatography, Yeast Two Hybrid Assay and/or Tandem Affinity Purification and *Gaussia princeps* luciferase protein complementation assay. HPV E6 and E7 proteins appear to have evolved various strategies to make use of the ubiquitin system to support the viral lifecycle [21][22][23]. In particular, to avoid apoptosis, E6 mainly targets p53 while, by targeting the pRb, p107 and p130 pocket proteins, E7 induces cell cycle progression [24][25][26]. E6 proteins from high-risk [27] and low-risk [28][29] HPVs are able to bind p53, and it was further shown that this binding promotes the degradation of p53 via the ubiquitin pathway [24]. Interestingly, E6 proteins from both high- and low-risk HPVs were also shown to have the capacity to bind p53 but without inducing its degradation [30][31]. Furthermore, a recent study showed that in the case of beta HPVs, HPV17a, HPV-38, and HPV-92 E6s could bind and/or stabilize p53 [32].

E6 and E7 oncoproteins inactivate the majority of these cellular substrates by interacting with components of the UPS, ultimately inducing their degradation at the proteasome. Among the diverse components of the UPS, the interplay between E6/E7 and ubiquitin ligases is one of the crucial aspects in this process. The best characterized ubiquitin ligases used by the two oncoproteins are E6-associated protein (E6AP) and the cullin-2 ubiquitin ligase complex [33]. E6AP complexes with E6 and is involved in targeting p53 and some other targets, while E7 uses the cullin-2 ubiquitin ligase complex to target pRb [24][34]. To date, this interaction has only been shown for HPV16 E7 [33]. There are also additional ubiquitin ligases and other components of the UPS, which are directed for similar activities by the viral oncoproteins [22][35][36][37][38][39]. Furthermore, HPV oncoprotein interactions with the UPS are not only critical for successful viral replication, but are also necessary for maintenance of the transformed phenotype [21][40]. Studies have shown that interference with components of the UPS has a down-regulatory effect on the ability of HPV-transformed cells to proliferate indefinitely and to avoid apoptosis [22][23][38][39][41]. Therefore, a better understanding of the interplay between HPVs and the UPS would provide important novel information and greatly improve the understanding of the viral life cycle and the process of HPV-induced tumorigenesis.

4. HPV E6 Oncoprotein and the UPS

The HPV E6 oncoprotein contains around 150 amino acids and has two zinc fingers created by four CXXC motifs [42][43][44]. The integrity of these motifs is crucial for optimal oncoprotein function and they are highly conserved between all HPV E6 oncoproteins identified so far [45][46]. Recent studies have characterized the crystal structure of the intact E6 oncoprotein and also support the fact that E6 forms interactions with a large number of cellular substrates [44][47][48][49][50]. Recent studies have characterized the crystal structure of the intact HPV-16 E6 oncoprotein and specific domains of HPV-18 E6, HPV-51 E6 and Bovine Papillomavirus 1 (BPV-1) E6, and these data all show that E6 forms interactions with a large number of cellular substrates [47][49][51]. HPV E6 complexes with these substrates through a number of conserved binding motifs. One of these is the so-called PDZ-binding motif (PBM), exclusively present on the C-terminus of HR HPV E6 oncoproteins, through which they bind to numerous PDZ-domain containing proteins [37][52]. Another conserved binding motif on E6 is the LXXLL binding motif. The most notable E6 targets with an LXXLL motif include E6AP, the preferred interacting partner of α-HPV E6 oncoproteins, and MAML-1, interacting with β-HPV E6 oncoproteins [53][54][55][56][57][58]. As mentioned above, E6 oncoproteins from both α- and β-HPV types interact with multiple components of the UPS; these interacting partners and their corresponding cellular functions are summarized in **Table 1**. By interacting with UPS components, HPV E6 oncoproteins can modulate various cellular functions, which are shown in **Figure 1.**

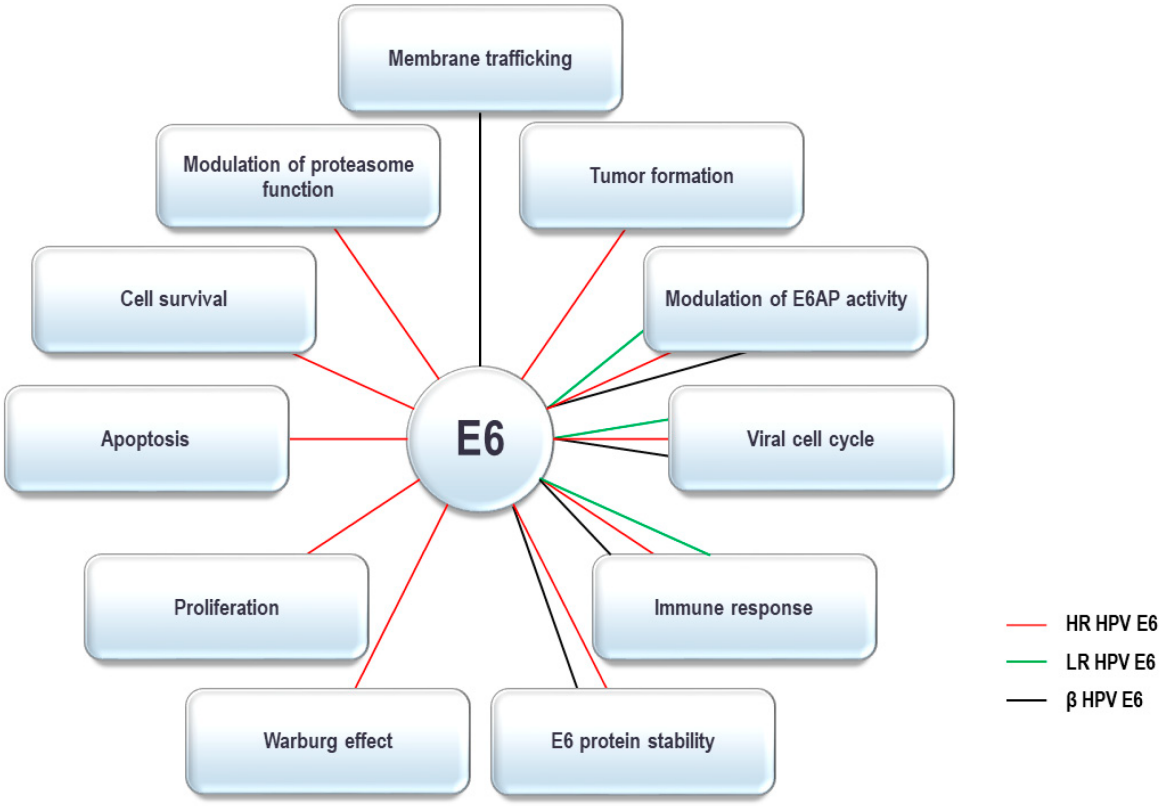

Figure 1. The Ubiquitin Proteasome System (UPS) dependent activities of α- and β-HPV E6 oncoproteins. E6 oncoproteins from α and β types interact with various components of the UPS and as speculated use them to modulate a number of cellular processes. By interacting with the UPS components high-risk (HR) α-type HPV E6s are involved in the regulation of all the processes shown above; low-risk (LR) α-type E6s are involved in regulation of viral life cycle, modulation of E6AP activity, and regulation of the immune response; β-type HPV E6s are involved in regulation of the viral life cycle, modulation of E6AP activity, regulation of the immune response, and E6 oncoprotein stability.

Table 1. HPV E6 interactions with ubiquitin-proteasome system components.

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
580	BARD1	Breast Cancer 1 Gene (BRCA1)-associated RING domain protein 1	Putative tumor suppressor gene mutated cancers. Homologous to BRCA1 RING motif and BRCT domain. BARD1/BRCA1 heterodimer is disrupted by tumorigenic amino acid substitutions in BRCA1. Heterodimer is required for BRCA1 tumor suppression and increases stability of both proteins.	−	+	?	Yeast-two hybrid, co-IP	Yim et al. (2007)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
672	BRCA1	Breast Cancer type 1 susceptibility protein	Tumor suppressor. The E3 ubiquitin-protein ligase component of BARD1/BRCA1 heterodimer. BRCA1/BARD1 heterodimer coordinates DNA damage repair, ubiquitination and transcriptional regulation to maintain genomic stability.	?	+	?	IP, GST-pull down	Zhang et al. (2005)
4850	CNOT4	CCR4-NOT transcription complex subunit 4	E3 ubiquitin-protein ligase, promoting degradation of target proteins. Involved in JAK/STAT activation	−	−	+	IP, MS	White et al. (2012)
1540	CYLD	CYLD lysine 63 (K63) DUB	A lysine 63 (K63) deubiquitinase. Tumor suppressor negatively regulating NF-κB pathway. Ubiquitinated and degraded during Hypoxia-induced NF-κB activation to relieve its inhibition of NF-κB signaling cascade	?	+	?		An et al. (2008)
8925	HERC1	HECT and RLD domain containing E3 ubiquitin protein ligase family member 1	An E3 ubiquitin-protein ligase accepts ubiquitin from an E2 ubiquitin-conjugating enzyme and then transfers the ubiquitin to targeted substrates. Involved in membrane trafficking.	−	−	+	IP, proximity ligation in situ assay	Holloway et al. (2015)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
8924	HERC2	HECT and RLD domain containing E3 ubiquitin-protein ligase 2	A putative HECT domain E3 ligase. Involved in protein trafficking and degradation pathways regulating ubiquitin-dependent retention of repair proteins on damaged chromosomes. Recruited to sites of DNA damage in response to ionizing radiation. Promotes DNA damage-induced formation of 'Lys-63'-linked ubiquitin chains.	<+	+	<+	IP, MS	Vos et al. (2009); White et al. (2012)
83737	ITCH	E3 ubiquitin-protein ligase Itchy	An E3 ubiquitin-protein ligase which is involved in the control of inflammatory signaling pathways. An essential component of an ubiquitin-editing protein complex ensuring the transience of inflammatory signaling pathways by regulating ubiquitin-dependent signaling events. Involved in the cellular antiviral response.	?	+	?	GPCA	Poirson et al. (2017)
23295	MGRN1	Mahogunin ring finger 1	Has RING- E3 ubiquitin-protein ligase activity in vitro. Involved in regulation of endosome-to-lysosome trafficking. A negative regulator of hedgehog signaling.	+	+	+	GPCA, co-IP	Poirson et al. (2017)
23024	PDZRN3	PDZ domain containing ring finger 3	A member of the LNX (Ligand of Numb Protein-X) family of RING E3 ubiquitin- protein ligases. Required for vascular morphogenesis and differentiation of adipocytes, osteoblasts and myoblasts.	−	+	+	GST-pull down	Poirson et al. (2017); Thomas and Banks (2015)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
29951	PDZRN4	PDZ domain containing ring finger 4	A member of the LNX family of RING E3 ubiquitin-protein ligases. A suppressor of cell proliferation in human liver cancer cell lines.	−	+	−	GPCA, co-IP	Poirson et al. (2017)
5684	PSMA3	Proteasome subunit alpha 3	Components of the 20S core proteasome complex involved in proteolysis of most cellular proteins. Associated with two 19S regulatory particles, form the 26S proteasome.	+	+	+	IP-MS/MS	White et al. (2012)
5695	PSMB7	Proteasome subunit beta 7		+	+	−	IP-MS/MS	White et al. (2012)
5698	PSMB9	Proteasome subunit beta 9	Involved in ATP-dependent degradation of ubiquitinated proteins.	−	+	−	IP-MS/MS	White et al. (2012)
5700	PSMC1	Proteasome 26S subunit 4, ATPase 1	A component of the 26S proteasome belonging to the heterohexameric ring of AAA proteins (ATPases associated with diverse cellular activities). Unfolds ubiquitinated target proteins.	?	+	?	GST-pull down	Tomaić et al. (2013)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
5701	PSMC2	Proteasome 26S subunit 7, ATPase 2		+	+	+	IP-MS/MS	White et al. (2012); Tomaić et al. (2013)
5702	PSMC3	Proteasome 26S regulatory subunit 6A, ATPase 3		+	+	−	IP-MS/MS, GST-pull donw	White et al. (2012); Tomaić et al. (2013)
5704	PSMC4	Proteasome 26S subunit 6B, ATPase 4	Components of the 26S proteasome.	+	+	−	IP-MS/MS	White et al. (2012)
5705	PSMC5	26S proteasome regulatory subunit 8		?	+	?	GST-pull down	Tomaić et al. (2013)
5707	PSMD1	26S proteasome non-ATPase regulatory subunit 1		+	+	+	IP-MS/MS	White et al. (2012)
5708	PSMD2	Proteasome 26S subunit 2, non-ATPase 2	Component of the 26S proteasome, binds to the intracellular domain of tumor necrosis factor type 1 receptor (TNFR1); the binding domain of TRAP1 and TRAP2 resides outside the death domain of TNFR1.	+	+	+	IP-MS/MS, GST-pull down	White et al. (2012); Tomaić et al. (2013)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
5710	PSMD4	26S proteasome non-ATPase regulatory subunit 4	A major ubiquitin-accepting proteasome subunit. Involved in maintaining structural integrity of the 19S regulatory particle. Important in direct and indirect recognition of ubiquitinated substrates of 26S proteasome by interacting with polyubiquitinated proteins and directing them to the proteasome for degradation. A critical controlling factor in regulation of protein degradation at the proteasome.	?	+	?	IP-MS/MS	Tomaić et al. (2013)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
5709	PSMD3	Proteasome 26S subunit 3, non-ATPase 3		+	+	+	IP-MS/MS	White et al. (2012)
9861	PSMD6	Proteasome 26S subunit, non-ATPase 6		+	+	−	IP-MS/MS	White et al. (2012)
5713	PSMD7	Proteasome 26S subunit, non-ATPase 7		+	+	−	IP-MS/MS	White et al. (2012)
5714	PSMD8	Proteasome 26S subunit, non-ATPase 8	Components of the 26S proteasome.	+	+	−	IP-MS/MS	White et al. (2012)
5719	PSMD13	26S proteasome non-ATPase regulatory subunit 13		+	+	+	IP-MS/MS	White et al. (2012)
10213	PSMD14	26S proteasome non-ATPase regulatory subunit 14		+	+	−	IP-MS/MS	White et al. (2012)
23198	PSME4	Proteasome activator subunit 4	A proteasome component that specifically recognizes and promotes ATP- and ubiquitin-independent degradation of acetylated core histones during DNA damage response to double-strand breaks.	−	+	−	IP-MS/MS	White et al. (2012)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
64320	RNF25	Ring finger protein 25	A RING finger-dependent E3 ubiquitin-protein ligase that mediates ubiquitination and stimulates transcription mediated by NF-κB.	?	+	?	GPCA	Poirson et al. (2017)
9810	RNF40	Ring finger protein 40	A component of the RNF20/40 E3 ubiquitin-protein ligase complex; forms a H2B ubiquitin ligase complex in cooperation with the UBE2A or UBE2B. Supports maintenance of tumorigenic features and inflammatory signaling by promoting nuclear NF-κB activity.	?	+	?	GPCA	Poirson et al. (2017)
85456	TNKS1BP1	Tankyrase-1-binding protein	A subunit of the smaller 1-MDa core of Ccr4-Not complex. Ccr4-Not is an mRNA deadenylase and has a ubiquitin-protein ligase function.	<+	<+	+	IP-MS/MS	White et al. (2012)
7188	TRAF5	TNF receptor associated factor 5	Tumor necrosis factor receptor-associated factor (TRAF) protein family. An adapter protein and signal transducer linked to various signaling pathways by association with the receptor cytoplasmic domain and kinases. Involved in cytokine signaling and mediates activation of NF-κB and JNK. It is also involved in apoptosis.	?	+	?	GPCA	Poirson et al. (2017)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
7189	TRAF6	TNF receptor-associated factor 6	An E3 ubiquitin-protein ligase that mediates the synthesis of 'Lys-63'-linked-polyubiquitin chains conjugated to target proteins and ubiquitination of unanchored poly-ubiquitin chains. Induces activation of NF-κB and JUN. An adaptor protein and signal transducer linking TNFR proteins to different signaling pathways. Plays a role in signal transduction initiated via TNF receptor, IL-1 receptor and IL-17 receptor.	?	+	?	GPCA	Poirson et al. (2017)
7706	TRIM25	Tripartite motif containing 25	An E3 ubiquitin-protein ligase involved in innate immune defense against viruses. Crucially involved in the interferon response to viral infection.	+	+	+	co-IP	Chiang et al. (2018)
10422	UBAC1	UBA domain containing 1	A ubiquitin-protein ligase required for poly-ubiquitination and proteasome-mediated degradation of CDKN1B during G1 phase of the cell cycle. A non-catalytic subunit of the KPC Kip1 ubiquitination- promoting complex.	?	+	?	GPCA	Poirson et al. (2017)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
7337	UBE3A	Ubiquitin-protein ligase E3A	An E3-HECT domain-containing ubiquitin-protein ligase. It promotes its own degradation in vivo. This imprinted gene is maternally expressed in the brain and biallelically expressed in other tissues. It plays an important role in regulation of the circadian clock and acts as a regulator of synaptic development.	<+	+	+	Yeast-two hybrid, co-crystal x-ray crystalography, co-IP	Poirson et al. (2017); Storey et al. (1998); Kao et al. (2000); Brimer et al., 2007; Thomas et al. (2013)
23352	UBR4	Ubiquitin-protein ligase E3 component n-recognin 4	An E3 ubiquitin-protein ligase, a component of the N-end rule pathway. Recognizes and binds to proteins bearing a specific N-terminal leading to ubiquitination and subsequent degradation. Forms meshwork structures involved in membrane morphogenesis and cytoskeletal organization and regulates integrin-mediated signaling.	−	−	+	IP	White et al. (2012); Thomas et al. (2013)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
51366	UBR5	Ubiquitin-protein ligase E3 component n-recognin 5	Also known as EDD (E3 identified by Differential Display). A HECT domain-containing E3 ubiquitin-protein ligase component of the N-end rule pathway. Involved in coordinating the balance between cell cycle progression and differentiation. A regulator of DNA damage response, acts as a guard against excessive spreading of ubiquitinated chromatin at damaged chromosomes, as well as tumor suppressor. Frequently overexpressed in breast and ovarian cancer.	−	+	+	co-IP, MS	Tomaić et al. (2011); White et al. (2012)
9958	USP15	Ubiquitin specific peptidase 15	A deubiquitinating enzyme of the ubiquitin specific protease (USP) family. Plays a critical role in ubiquitin-dependent processes through polyubiquitin chain disassembly and hydrolysis of ubiquitin-substrate bonds.	−	+	+	Yeast-two hybrid, GPCA, IP	Vos et al. (2009); Poirson et al. (2017); Yaginuma et al. (2018); Chiang et al. (2018)
64854	USP46	Ubiquitin carboxyl-terminal hydrolase 46	A deubiquitinating enzyme that plays a role in behavior by regulating GABA action. Has little intrinsic deubiquitinating activity and requires interaction with regulator of deubiquitinating complexes WDR48 (WD repeat-containing protein 48) for high activity.	−	+	?	co-IP	Kiran et al. (2018)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
7428	VHL	Von Hippel-Lindau tumor suppressor	VHL is a component of the protein complex that includes elongin C, elongin B, and cullin-2. An E3 ubiquitin-protein ligase involved in the ubiquitination and degradation of hypoxia-inducible factor (HIF), a transcription factor crucial to oxygen-related gene expression.. HPV16 E6 promotes hypoxia-induced Warburg effect through blocking the association of HIF-1α and VHL	?	+	?	GPCA	Poirson et al. (2017); Guo et al. (2014)
331	XIAP	X-linked inhibitor of apoptosis	A multi-functional protein that regulates apoptosis, modulates inflammatory signaling and immunity, cell proliferation, cell invasion and metastasis. An E3 ubiquitin-protein ligase regulating NF-κB signaling and other target. An important regulator of innate immune signaling via regulation of Nod-like receptors (NLRs).	?	+	?	GPCA	Poirson et al. (2017)

+ confirmed to interact; +, < confirmed interactions with lower affinity compared to hR-HPV E6; - interactions not detected; ? interactions not tested. Co-IP: co-immunoprecipitation; GPCA: Gaussia princeps luciferase protein complementation assay, IP-MS/MS: immunoprecipitation-mass spectrometry, IP: immunoprecipitation.

5. HPV E7 Oncoprotein and the UPS

HPV E7 oncoprotein is a small acidic protein of approximately 100 amino acid residues, having no significant sequence similarities to any cellular protein, except for the LXCXE motif. The amino terminus contains two regions similar to the E1A adenovirus proteins: the conserved region 2 (CR2) and part of CR1 [42]. The E7 oncoprotein also contains CR3, a zinc-binding site formed by two CXXC domains that function as a dimerization domain [59]. The CR3 domains are similar to those of the HPV E6 protein, suggesting that a genetic duplication may have occurred early in HPV evolution [60]. E7 has, in addition, sequences related to those of simian vacuolating virus 40 large tumor antigen (SV40 T), all contributing to the transforming activities of HR HPV E7 oncoproteins [61][62][63][64].

The main function of the HPV E7 oncoprotein is to maintain the infected differentiating cell in a DNA replication-competent state, which it does in part, by targeting the retinoblastoma tumor suppressor (pRb), a critical regulator of cell cycle progression, which controls the G1 to S-phase transition [65]. E7 also binds to the pRb-related members of the pocket

protein family, p107 and p130, which assists in driving the cell cycle of the differentiating HPV-infected epithelial cell into an S-phase-like state, an environment suitable for replication of the viral genome [66]. In order to complete these activities, E7 forms interactions with ubiquitin ligases, which are essential elements of the UPS. In addition, E7 was shown to complex with other components of the UPS, and these interactions appeared to be important either for the stability of the oncoprotein or for its role in regulation and modulation of many cellular processes, some of which are summarized in **Table 2** and **Figure 2**.

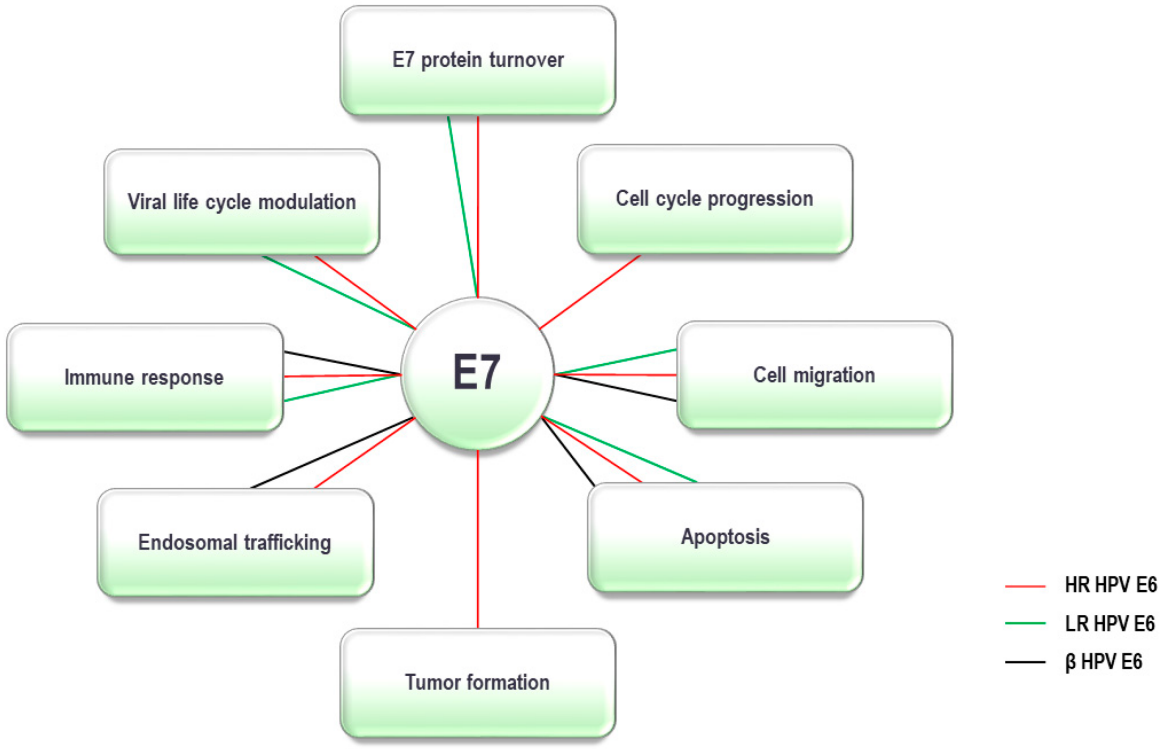

Figure 2. The Ubiquitin Proteasome System (UPS) dependent activities of α- and β-HPV E7 oncoproteins. E7 oncoproteins from α and β types interact with various components of the UPS and are thought to use them to modulate a number of cellular processes. By interacting with the UPS components, high-risk (HR) α-type E7s are involved in the regulation of all the processes shown above; low-risk (LR) α-type E7s are involved in cell migration, apoptosis, regulation of the immune response, viral life cycle modulation and E7 protein turnover; β-type E7s are involved in cell migration, apoptosis, endosomal trafficking and regulation of the immune response.

Table 2. HPV E7 interactions with ubiquitin-proteasome system components.

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
8454	CUL1	Cullin-1	A core component of cullin-RING-based SCF E3 ubiquitin- protein ligase complex, which mediates ubiquitination and proteolysis of E7.	+	+	?	IP	Münger et al. (1989); Boyer et al. (1996); Oh et al. (2004)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
8453	CUL2	Cullin-2	A core component of cullin-RING-based ECS E3 ubiquitin- protein ligase complex. Stabilizes APOBEC3A. Required for E7-induced degradation of pRB contributing to cell transformation by dysregulating G1/S cell cycle checkpoints	−	+	-	co-IP	Huh et al. (2007); Narisawa-Saito and Kiyono. (2007); White et al. (2012); Xu et al. (2016); Westrich et al. (2018)
8452	CUL3	Cullin-3	A core component of cullin-RING-based BCR E3 ubiquitin- protein ligase complexes, which mediate the ubiquitination and proteasomal degradation of target proteins.	+	+	?	co-IP	White et al. (2012); Poirson et al. (2017)
90379	DCAF15	DDB1 and CUL4 associated factor 15	May be involved in ubiquitination and degradation through a DBB1-CUL4 E3 protein-ubiquitin ligase.	−	+	−	GPCA, co-IP	Poirson et al. (2017)
253980	KCTD13	Potassium channel tetramer-ization domain 13	A substrate-specific adapter of a BCR E3 ubiquitin-protein ligase complex required for synaptic transmission.	+	+	+	GPCA, co-IP	Chen et al. (2009); Poirson et al. (2017)
112939	NACC1	Nucleus accumbens-associated protein 1	A transcriptional repressor and transcriptional corepressor in neuronal cells through recruitment of HDAC3 and HDAC4 Required to recruit the proteasome from the nucleus to the cytoplasm and dendritic spines.	?	+	?	GPCA	Poirson et al. (2017)
9148	NEURL1	Neuralized E3 ubiquitin-protein ligase 1	An E3 ubiquitin- protein ligase that activates in vitro ubiquitination of JAG1, inhibiting malignant cell transformation of medulloblastoma cells through the Notch pathway.	+	+	+	GPCA, co-IP	Poirson et al. (2017)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
5700	PSMC1	26S proteasome regulatory subunit 4	A component of the 26S proteasome.	−	+	?	IP	Berezutskaya and Bagchi (1997); Ben-Saadon et al. (2004)
84282	RNF135	ring finger protein 135	An E2-dependent E3 ubiquitin-protein ligase, involved in innate immune defense against viruses.	+, <	+	−	GPCA, co-IP	Poirson et al. (2017)
57630	SH3RF1	SH3 domain containing ring finger 1	Has an E3 ubiquitin-protein ligase activity. In the absence of an external substrate, it can catalyze self-ubiquitination.	+, <	+	?	GPCA	Poirson et al. (2017)
92799	SHKBP1	SH3KBP1-binding protein 1	SHKBP1 inhibits CBL-SH3KBP1 complex mediated downregulation of EGFR signaling by sequestration of SH3KBP1.	+	+	+	GPCA, co-IP	Poirson et al. (2017)
7126	TNFAIP1	TNF alpha induced protein 1	A substrate-specific adapter of a BCR E3 ubiquitin-protein ligase complex that mediates the ubiquitination and proteasomal degradation of RhoA, thereby regulating the actin cytoskeleton and cell migration. HPV-16 E7 can modulate the responses of its natural host cell to the closely related cytokines TNF-α.	+	+	+	GPCA, co-IP	Basile et al. (2001); Poirson et al. (2017)
7186	TRAF2	TNF receptor associated factor 2	Regulates the activation of NF-κB and JNK and plays a central role in the regulation of cell survival and apoptosis. An essential constituent of several E3 ubiquitin-protein ligases. HPV16 E6/E7 switch cells from apoptotic to proliferative fates under TWEAK/Fn14 interaction, possibly by favoring Ras and TRAF2 activation and modulating TNF receptor expression.	−	+	+	GPCA, co-IP	Cheng et al. (2015); Poirson et al. (2017)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
7187	TRAF3	TNF receptor associated factor 3	Regulates pathways leading to activation of NF-κB and MAP kinases, and plays a central role in the regulation of B-cell survival. An essential constituent of several E3 ubiquitin-protein ligase complexes. Overexpression of TRAF3 enhances p53 and pRb expression	?	+	?	GPCA	Poirson et al. (2017); Zhang et al. (2018)
9618	TRAF4	TNF receptor associated factor 4	An adaptor protein and signal transducer linking members of the TNFR family to different signaling pathways. Plays a role in the activation of NF-κB and JNK, and in the regulation of cell survival and apoptosis. May interact selectively and non-covalently with E3 ubiquitin- protein ligase enzymes.	?	+	?	GPCA	Poirson et al. (2017)
7188	TRAF5	TNF receptor associated factor 5	An adaptor protein and signal transducer linking members of the TNFR family to different signaling pathways. May interact selectively and non-covalently with E3 ubiquitin-protein ligase enzymes.	+	+	+	GPCA, co-IP	Poirson et al. (2017)
10346	TRIM22	tripartite motif containing 22	An interferon- induced antiviral protein involved in innate immunity. May have E3 ubiquitin-protein ligase activity. Activated by integration of E6/E7 genes.	?	+	?	GPCA	Pett et al. (2006); Poirson et al. (2017)
22954	TRIM32	tripartite motif containing 32	An E3 ubiquitin-protein ligase. It ubiquitinates DTNBP1 and promotes its degradation.	+	+	+	GPCA, co-IP	Poirson et al. (2017)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
493829	TRIM72	tripartite motif containing 72	A muscle-specific protein that plays a central role in cell membrane repair by nucleating the assembly of the repair machinery at injury sites. May be involved in proteasome- mediated, ubiquitin-dependent protein catabolic processes.	−	+	+	GPCA, co-IP	Poirson et al. (2017)
114088	TRIM9	tripartite motif containing 9	An E3 ubiquitin- protein ligase, which self-ubiquitinates in cooperation with E2 enzyme UBE2D2/UBC4. Serves as a targeting signal for proteasomal degradation.	?	+	?	GPCA	Poirson et al. (2017)
7314	UBB	ubiquitin B	Targets cellular proteins for degradation by the 26S proteasome. Involved in the maintenance of chromatin structure, the regulation of gene expression, and the stress response.	?	+	?	GPCA	Poirson et al. (2017)
7319	UBE2A	Ubiquitin-conjugating enzyme E2A	Accepts ubiquitin from the E1 complex. In association with the E3 enzyme, UBE2A plays a role in transcription regulation by catalyzing the ubiquitination of histone H2B.	+	+	?	GPCA	Poirson et al. (2017)
7332	UBE2L3	Ubiquitin-conjugating enzyme E2L3	Specifically acts with HECT-type and RBR family E3 ubiquitin- protein ligases. Accepts ubiquitin from the E1 complex and catalyzes its covalent attachment to other proteins including E7.	?	+	?	IP	Reinstein et al. (2000)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
23352	UBR4	Ubiquitin-protein ligase E3 component n-recognin 4	Also known as p600. An E3 ubiquitin- protein ligase that recognizes proteins with specific destabilized N-terminal residues, leading to their ubiquitination and degradation. May mediate some pRB- independent transforming activities of HPV-16 E7, but is not sufficient for cellular transformation as interactions were also found with low-risk HPV E7 oncoproteins. It is speculated that UBR4 could potentially play a role in viral replication.	+	+	+	IP	Huh et al. (2005); De Masi et al. (2005); White et al. (2012)
8237	USP11	Ubiquitin carboxyl-terminal hydrolase 11	Inhibits degradation of target proteins by the proteasome. Plays a role in the regulation of pathways leading to NF-κ-B activation. Augments HPV-16E7 activity in modulating downstream target genes, such as pRb, Bcl-2, and Cdc-2, suggesting that this interaction may contribute to cell transformation by HPV-16E7.	?	+	?	IP	Lin et al. (2008); Poirson et al. (2017)
83844	USP26	Ubiquitin carboxyl-terminal hydrolase 26	Involved in the ubiquitin-dependent proteolytic pathway in conjunction with the 26S proteasome.	+	+	+	GPCA, co-IP	Poirson et al. (2017)
57663	USP29	Ubiquitin carboxyl-terminal hydrolase 29	A thiol-dependent hydrolyser of ester, thioester, amide, peptide and isopeptide bonds formed by the C-terminal Gly of ubiquitin.	−	+	−	GPCA, co-IP	Poirson et al. (2017)
23032	USP33	Ubiquitin carboxyl-terminal hydrolase 33	A deubiquitinating enzyme involved in centrosome duplication, cell migration and beta-2 adrenergic receptor/ADRB2 recycling.	+	+	+	GPCA, co-IP	Poirson et al. (2017)

Gene ID	Gene	Protein	Function	α-HPV		β-HPV	Method	Ref.
				LR	HR			
90850	ZNF598	Zinc finger protein 598	An E3 ubiquitin- protein ligase required for terminal stalling of ribosomes during translation of poly(A) sequences by mediating ubiquitination of 40S ribosomal protein.	?	+	?	GPCA	Poirson et al. (2017)

+ confirmed to interact; +, < confirmed interactions with lower affinity compared to HR-HPV E7; - interactions not detected; ? interactions not tested. Co-IP: co-immunoprecipitation; GPCA: *Gaussia princeps luciferase* protein complementation assay, IP-MS/MS: immunoprecipitation-mass spectrometry, IP: immunoprecipitation.

References

1. International Agency for Cancer Research. IARC Monographs 100B—Human Papillomaviruses 2012; International Agency for Cancer Research, IARC Press: Lyon, France, 2005.
2. King, A.M.Q.; Adams, M.J.; Carstens, E.B.; Lefkowitz, E.J. Virus Taxonomy: Classification and Nomenclature of Viruses. Virus Taxon. 2005.
3. López-Bueno, A.; Mavian, C.; Labella, A.M.; Castro, D.; Borrego, J.J.; Alcami, A.; Alejo, A. Concurrence of Iridovirus, Polyomavirus, and a Unique Member of a New Group of Fish Papillomaviruses in Lymphocystis Disease-Affected Gilthead Sea Bream. J. Virol. 2016, 90, 8768–8779.
4. de Villiers, E.-M.; Burk, R.D.; Bernard, H.-U.; Chen, Z.; van Doorslaer, K.; zur Hausen, H. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 2010, 401, 70–79.
5. Doorbar, J. The papillomavirus life cycle. J. Clin. Virol. 2005, 32 (Suppl. S1), S7–S15.
6. Moody, C.A.; Laimins, L.A. Human papillomavirus oncoproteins: Pathways to transformation. Nat. Rev. Cancer 2010, 10, 550–560.
7. Morgan, E.L.; Wasson, C.W.; Hanson, L.; Kealy, D.; Pentland, I.; McGuire, V.; Roberts, S. STAT3 activation by E6 is essential for the differentiation-dependent HPV18 life cycle. PLoS Pathog. 2018, 14.
8. Hershko, A.; Ciechanover, A. The ubiquitin system. Annu. Rev. Biochem. 1997, 425–479.
9. Ciechanover, A. Intracellular protein degradation: From a Vague Idea, through the lysosome and the ubiquitin-proteasome system, and onto human diseases and drug targeting (Nobel Lecture). Angew. Chem. Int. Ed. 2005, 44, 5944–5967.
10. Tanaka, K. The proteasome: Overview of structure and functions. Proc. Jpn. Acad. Ser. B 2009, 85, 12–36.
11. Rousseau, A.; Bertolotti, A. Regulation of proteasome assembly and activity in health and disease. Nat. Rev. Mol. Cell Biol. 2018, 19, 697–712.
12. Saeki, Y. Ubiquitin recognition by the proteasome. J. Biochem. 2017, 161, 113–124.
13. Finley, D. Recognition and processing of ubiquitin-protein conjugates by the proteasome. Annu. Rev. Biochem. 2009, 78, 477–513.
14. Poirson, J.; Biquand, E.; Straub, M.-L.; Cassonnet, P.; Nominé, Y.; Jones, L.; Demeret, C. Mapping the interactome of HPV E6 and E7 oncoproteins with the ubiquitin-proteasome system. FEBS J. 2017, 284, 3171–3201.
15. Ingham, R.J.; Gish, G.; Pawson, T. The Nedd4 family of E3 ubiquitin ligases: Functional diversity within a common modular architecture. Oncogene 2004, 23, 1972–1984.
16. Li, W.; Bengtson, M.H.; Ulbrich, A.; Matsuda, A.; Reddy, V.A.; Orth, A.; Joazeiro, C.A. Genome-wide and functional annotation of human E3 ubiquitin ligases identifies MULAN, a mitochondrial E3 that regulates the organelle's dynamics and signaling. PLoS ONE 2008, 3, e1487.
17. Li, W.; Ye, Y. Polyubiquitin chains: Functions, structures, and mechanisms. Cell. Mol. Life Sci. 2008, 65, 2397–2406.

18. Rotin, D.; Kumar, S. Physiological functions of the HECT family of ubiquitin ligases. Nat. Rev. Mol. Cell Biol. 2009, 10, 398–409.

19. Wing, S.S. Deubiquitinating enzymes-the importance of driving in reverse along the ubiquitin-proteasome pathway. Int. J. Biochem. Cell Biol. 2003, 35, 590–605.

20. Tomaić, V.; Banks, L. Angelman syndrome-associated ubiquitin ligase UBE3A/E6AP mutants interfere with the proteolytic activity of the proteasome. Cell Death Dis. 2015, 6, e1625.

21. Lou, Z.; Wang, S. E3 ubiquitin ligases and human papillomavirus-induced carcinogenesis. J. Int. Med. Res. 2014, 42, 247–260.

22. Vos, R.M.; Altreuter, J.; White, E.A.; Howley, P.M. The Ubiquitin-Specific Peptidase USP15 Regulates Human Papillomavirus Type 16 E6 Protein Stability. J. Virol. 2009, 83, 8885–8892.

23. Tomaić, V.; Pim, D.; Banks, L. The stability of the human papillomavirus E6 oncoprotein is E6AP dependent. Virology 2009, 393, 7–10.

24. Scheffner, M.; Werness, B.A.; Huibregtse, J.M.; Levine, A.J.; Howley, P.M. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell 1990, 63, 1129–1136.

25. Helt, A.-M.; Funk, J.O.; Galloway, D.A. Inactivation of both the Retinoblastoma Tumor Suppressor and p21 by the Human Papillomavirus Type 16 E7 Oncoprotein Is Necessary To Inhibit Cell Cycle Arrest in Human Epithelial Cells. J. Virol. 2002, 76, 10559–10568.

26. Münger, K.; Werness, B.A.; Dyson, N.; Phelps, W.C.; Harlow, E.; Howley, P.M. Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. EMBO J. 1989, 8, 4099–4105.

27. Werness, B.A.; Levine, A.J.; Howley, P.M. Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science 1990, 248, 76–79.

28. Pietsch, E.C.; Murphy, M.E. Low risk HPV-E6 traps p53 in the cytoplasm and induces p53-dependent apoptosis. Cancer Biol. Ther. 2008, 7, 1916–1918.

29. Oh, S.T.; Longworth, M.S.; Laimins, L.A. Roles of the E6 and E7 Proteins in the Life Cycle of Low-Risk Human Papillomavirus Type 11. J. Virol. 2004, 78, 2620–2626.

30. Crook, T.; Tidy, J.A.; Vousden, K.H. Degradation of p53 can be targeted by HPV E6 sequences distinct from those required for p53 binding and trans-activation. Cell 1991, 67, 547–556.

31. Lechner, M.S.; Laimins, L.A. Inhibition of p53 DNA binding by human papillomavirus E6 proteins. J. Virol. 1994, 68, 4262–4273.

32. White, E.A.; Walther, J.; Javanbakht, H.; Howley, P.M. Genus beta human papillomavirus E6 proteins vary in their effects on the transactivation of p53 target genes. J. Virol. 2014, 88, 8201–8212.

33. White, E.A.; Sowa, M.E.; Tan, M.J.A.; Jeudy, S.; Hayes, S.D.; Santha, S.; Münger, K.; Harper, J.W.; Howley, P.M. Systematic identification of interactions between host cell proteins and E7 oncoproteins from diverse human papillomaviruses. Proc. Natl. Acad. Sci. USA 2012, 109, E260–E267.

34. Huh, K.; Zhou, X.; Hayakawa, H.; Cho, J.-Y.; Libermann, T.A.; Jin, J.; Harper, J.W.; Munger, K. Human Papillomavirus Type 16 E7 Oncoprotein Associates with the Cullin 2 Ubiquitin Ligase Complex, Which Contributes to Degradation of the Retinoblastoma Tumor Suppressor. J. Virol. 2007, 81, 9737–9747.

35. Szalmás, A.; Tomaić, V.; Basukala, O.; Massimi, P.; Mittal, S.; Kónya, J.; Banks, L. The PTPN14 Tumor Suppressor Is a Degradation Target of Human Papillomavirus E7. J. Virol. 2017, 91.

36. Holloway, A.; Simmonds, M.; Azad, A.; Fox, J.L.; Storey, A. Resistance to UV-induced apoptosis by β-HPV5 E6 involves targeting of activated BAK for proteolysis by recruitment of the HERC1 ubiquitin ligase. Int. J. Cancer 2015, 136, 2831–2843.

37. Bennett Saidu, N.E.; Filić, V.; Thomas, M.; Sarabia-Vega, V.; Đukić, A.; Miljković, F.; Banks, L.; Tomaić, V. PDZ Domain-Containing Protein NHERF-2 is a Novel Target of Human Papillomavirus type 16 (HPV-16) and HPV-18. J. Virol. 2019.

38. Huh, K.-W.; DeMasi, J.; Ogawa, H.; Nakatani, Y.; Howley, P.M.; Münger, K. Association of the human papillomavirus type 16 E7 oncoprotein with the 600-kDa retinoblastoma protein-associated factor, p600. Proc. Natl. Acad. Sci. USA 2005, 102, 11492–11497.

39. DeMasi, J.; Huh, K.-W.; Nakatani, Y.; Münger, K.; Howley, P.M. Bovine papillomavirus E7 transformation function correlates with cellular p600 protein binding. Proc. Natl. Acad. Sci. USA 2005, 102, 11486–11491.

40. Tomaić, V. Functional Roles of E6 and E7 Oncoproteins in HPV-Induced Malignancies at Diverse Anatomical Sites. Cancers 2016, 8, 95.

41. Grm, H.S.; Banks, L. Degradation of hDlg and MAGIs by human papillomavirus E6 is E6-AP-independent. J. Gen. Virol. 2004, 85, 2815–2819.

42. Cole, S.T.; Danos, O. Nucleotide sequence and comparative analysis of the human papillomavirus type 18 genome. Phylogeny of papillomaviruses and repeated structure of the E6 and E7 gene products. J. Mol. Biol. 1987, 193, 599–608.

43. Barbosa, M.S.; Wettstein, F.O. Transcription of the cottontail rabbit papillomavirus early region and identification of two E6 polypeptides in COS-7 cells. J. Virol. 1987, 61, 2938–2942.

44. Martinez-Zapien, D.; Ruiz, F.X.; Poirson, J.; Mitschler, A.; Ramirez, J.; Forster, A.; Cousido-Siah, A.; Masson, M.; Pol, S.V.; Podjarny, A.; et al. Structure of the E6/E6AP/p53 complex required for HPV-mediated degradation of p53. Nature 2016, 529, 541–545.

45. Kanda, T.; Watanabe, S.; Zanma, S.; Sato, H.; Furuno, A.; Yoshiike, K. Human papillomavirus type 16 E6 proteins with glycine substitution for cysteine in the metal-binding motif. Virology 1991, 185, 536–543.

46. Sherman, L.; Schlegel, R. Serum- and calcium-induced differentiation of human keratinocytes is inhibited by the E6 oncoprotein of human papillomavirus type 16. J. Virol. 1996, 70, 3269–3279.

47. Nominé, Y.; Masson, M.; Charbonnier, S.; Zanier, K.; Ristriani, T.; Deryckère, F.; Sibler, A.P.; Desplancq, D.; Atkinson, R.A.; Weiss, E.; et al. Structural and functional analysis of E6 oncoprotein: Insights in the molecular pathways of human papillomavirus-mediated pathogenesis. Mol. Cell. 2006, 21, 665–678.

48. Zanier, K.; Ruhlmann, C.; Melin, F.; Masson, M.; Ould M'hamed Ould Sidi, A.; Bernard, X.; Fischer, B.; Brino, L.; Ristriani, T.; Rybin, V.; et al. E6 proteins from diverse papillomaviruses self-associate both in vitro and in vivo. J Mol Biol 2010, 396, 90–104.

49. Zanier, K.; Charbonnier, S.; Sidi, A.O.M.O.; McEwen, A.G.; Ferrario, M.G.; Poussin-Courmontagne, P.; Cura, V.; Brimer, N.; Babah, K.O.; Ansari, T.; et al. Structural basis for hijacking of cellular LxxLL motifs by papillomavirus E6 oncoproteins. Science 2013, 339, 694–698.

50. Pim, D.; Banks, L. Interaction of viral oncoproteins with cellular target molecules: Infection with high-risk vs low-risk human papillomaviruses. APMIS 2010, 118, 471–493.

51. Zhang, Y.; Dasgupta, J.; Ma, R.Z.; Banks, L.; Thomas, M.; Chen, X.S. Structures of a human papillomavirus (HPV) E6 polypeptide bound to MAGUK proteins: Mechanisms of targeting tumor suppressors by a high-risk HPV oncoprotein. J. Virol. 2007, 81, 3618–3626.

52. Thomas, M.; Narayan, N.; Pim, D.; Tomaić, V.; Massimi, P.; Nagasaka, K.; Banks, L. Human papillomaviruses, cervical cancer and cell polarity. Oncogene 2008, 27, 7018–7030.

53. Huibregtse, J.M.; Scheffner, M.; Howley, P.M. Localization of the E6-AP regions that direct human papillomavirus E6 binding, association with p53, and ubiquitination of associated proteins. Mol. Cell. Biol. 1993, 13, 4918–4927.

54. Elston, R.C.; Napthine, S.; Doorbar, J. The identification of a conserved binding motif within human papillomavirus type 16 E6 binding peptides, E6AP and E6BP. J. Gen. Virol. 1998, 79 Pt 2, 371–374.

55. White, E.A.; Kramer, R.E.; Tan, M.J.A.; Hayes, S.D.; Harper, J.W.; Howley, P.M. Comprehensive Analysis of Host Cellular Interactions with Human Papillomavirus E6 Proteins Identifies New E6 Binding Partners and Reflects Viral Diversity. J. Virol. 2012, 86, 13174–13186.

56. Brimer, N.; Lyons, C.; Wallberg, A.E.; Vande Pol, S.B. Cutaneous papillomavirus E6 oncoproteins associate with MAML1 to repress transactivation and NOTCH signaling. Oncogene 2012, 31, 4639–4646.

57. Tan, M.J.A.; White, E.A.; Sowa, M.E.; Harper, J.W.; Aster, J.C.; Howley, P.M. Cutaneous β-human papillomavirus E6 proteins bind Mastermind-like coactivators and repress Notch signaling. Proc. Natl. Acad. Sci. USA 2012, 109, E1473–E1480.

58. Meyers, J.M.; Spangle, J.M.; Munger, K. The human papillomavirus type 8 E6 protein interferes with NOTCH activation during keratinocyte differentiation. J. Virol. 2013, 87, 4762–4767.

59. Clemens, K.E.; Brent, R.; Gyuris, J.; Munger, K. Dimerization of the human papillomavirus E7 oncoprotein in vivo. Virology 1995, 214, 289–293.

60. Münger, K.; Grace, M.; Yee, C.; Jones, D.L.; Mavromatis, K.O.; Mukherjee, R. The carboxyl-terminal zinc-binding domain of the human papillomavirus E7 protein can be functionally replaced by the homologous sequences of the E6 protein. Virus Res. 2002, 52, 109–118.

61. Phelps, W.C.; Yee, C.L.; Münger, K.; Howley, P.M. The human papillomavirus type 16 E7 gene encodes transactivation and transformation functions similar to those of adenovirus E1A. Cell 1988, 53, 539–547.

62. Phelps, W.C.; Munger, K.; Yee, C.L.; Barnes, J.A.; Howley, P.M. Structure-Function Analysis of the Human Papillomavirus Type 16 E7 Oncoprotein. J. Virol. 1992, 66, 2418–2427.
63. McLaughlin, M.; Münger, K. The Human Papillomavirus E7 Oncoprotein. Virology 2010, 384, 335–344.
64. Roman, A.; Munger, K. The papillomavirus E7 proteins. Virology 2013, 445, 138–168.
65. Smotkin, D.; Wettstein, F.O. Transcription of human papillomavirus type 16 early genes in a cervical cancer and a cancer-derived cell line and identification of the E7 protein. Proc. Natl. Acad. Sci. USA 1986, 83, 4680–4684.
66. Morris, E.J.; Dyson, N.J. Retinoblastoma protein partners. Adv. Cancer Res. 2001, 82, 1–54.