



Review

Hallmarks of HPV carcinogenesis: The role of E6, E7 and E5 oncoproteins in cellular malignancy



Diogo Estêvão^{a,b}, Natália Rios Costa^a, Rui M. Gil da Costa^{a,d}, Rui Medeiros^{a,b,c,e,*}

^a Molecular Oncology and Viral Pathology Group, IPO-Porto Research Center (CI-IPOP), Portuguese Institute of Oncology of Porto (IPO-Porto), Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

^b FMUP, Faculty of Medicine, University of Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal

^c LPCC, Research Department Portuguese League Against Cancer (Liga Portuguesa Contra o Cancro—Núcleo Regional do Norte), Estrada Interior da Circunvalação, no. 6657, 4200-177 Porto, Portugal

^d Center for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro, UTAD, Quinta de Prados, 5000-911 Vila Real, Portugal

^e CEBIMED, Faculty of Health Sciences, Fernando Pessoa University, Porto, Portugal

ARTICLE INFO

Keywords:

HPV
E6
E7 and E5 oncoproteins
HPV-induced cancers
Carcinogenesis

ABSTRACT

Human papillomavirus (HPV) is the most common sexually transmitted infectious agent worldwide, being also responsible for 5% of all human cancers. The integration and hypermethylation mechanisms of the HPV viral genome promote the unbalanced expression of the E6, E7 and E5 oncoproteins, which are crucial factors for the carcinogenic cascade in HPV-induced cancers. This review highlights the action of E6, E7 and E5 over key regulatory targets, promoting all known hallmarks of cancer. Both well-characterized and novel targets of these HPV oncoproteins are described, detailing their mechanisms of action. Finally, this review approaches the possibility of targeting E6, E7 and E5 for therapeutic applications in the context of cancer.

1. HPV biology

Human papillomavirus (HPV) is among the most prominent risk factors for cancer [1]. Since its identification as the cause of cervical cancer, this agent has been an intensive focus of research [2] (Fig. 1).

HPV itself is the most common sexually transmitted agent worldwide, afflicting 50–80% of the sexually active human population [3]. It is also responsible for one-third of all the tumours induced by viruses and accounts for 5% of all human cancers [3]. This translates to approximately 630,000 new HPV-related cancer cases per year, 570,000 of which occur in women and 60,000 in men [3]. While the incidence of cervical cancer has been decreasing, presumably due to the implementation of prophylactic vaccination and effective screening programs, those of anogenital and head-and-neck HPV-positive tumours are increasing [4–6]. According to the International Agency for Research on Cancer (IARC), HPV is the cause of 8500 annual cases of vulvar cancer, 12,000 cases of vaginal cancer, 35,000 cases of anal cancer and 13,000 cases of penile cancer [5]. Furthermore, per year, there are approximately 38,000 head and neck squamous cell carcinomas (HNSCCs) attributable to HPV, particularly in the oropharyngeal

region. An increase of 225% of HPV-positive oropharyngeal squamous cell carcinomas (OPSCC) from 1998 to 2004 has been noted, along with a regression of 50% of those cancers related to alcohol and tobacco [7]. HPV is expected to become the main etiological factor for Oropharyngeal Squamous Cell Carcinoma (OSCC) by 2040 [7,8].

HPV belongs to the Papillomaviridae family, since it is a small, double-stranded and non-enveloped virus with 50–55 nm in diameter [9]. Their episomal genome has approximately 8000 base pairs, mostly encoding eight open reading frames (ORFs), expressed from polycistronic messenger ribonucleic acid (mRNA) [9]. HPV infects the basal keratinocytes, and its expression is temporal and synchronised with their differentiation (Fig. 2) [10]. The virus expresses the “early” E1, E2, E4, E5, E6 and E7 proteins as well as the “late” major and minor capsid proteins L1 and L2 [11]. A long control region (LCR) is essential for regulating the viral expression [12].

Papillomaviruses are highly heterogeneous and more than 300 genotypes of papillomavirus (PVs) have already been identified, of which approximately 200 are capable of infecting humans [1]. HPV is grouped within five evolutionary branches, namely the α, β, γ, μ, and ν papillomaviruses. The largest HPV group, the α-HPV, which comprises

* Corresponding author at: Molecular Oncology and Viral Pathology Group, IPO-Porto Research Center (CI-IPOP), Portuguese Institute of Oncology of Porto (IPO-Porto), Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal.

E-mail address: ruimedei@ipoporto.min-saude.pt (R. Medeiros).

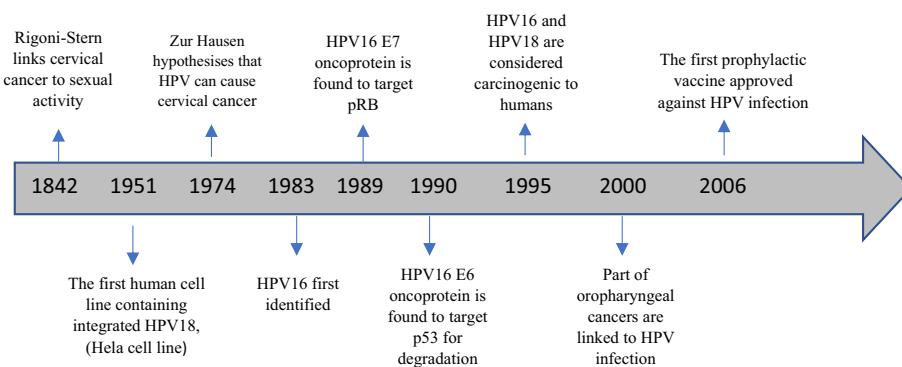


Fig. 1. Main milestones concerning HPV pathology.

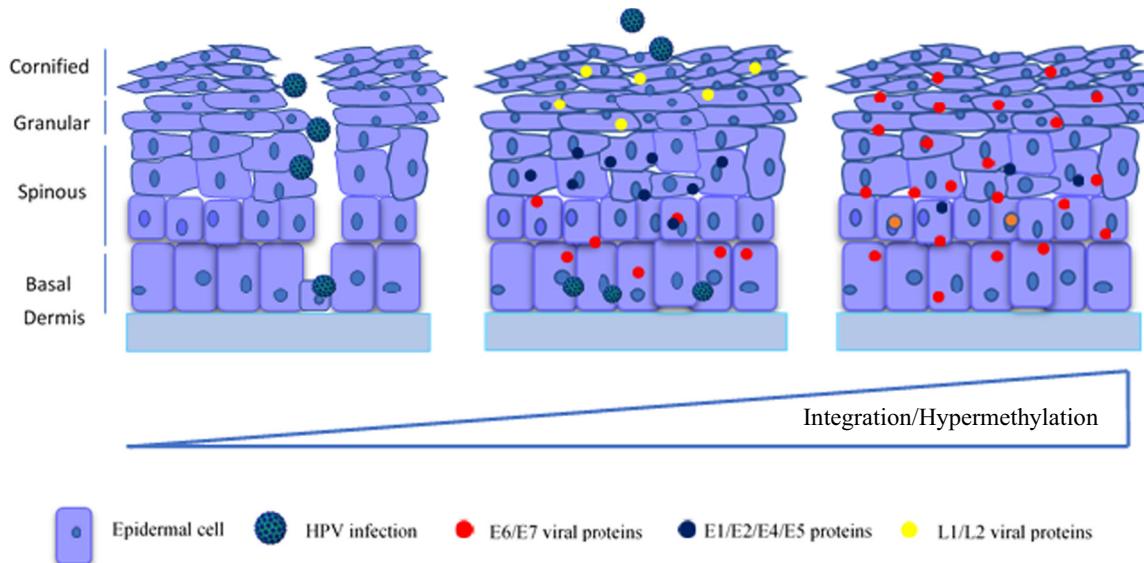


Fig. 2. HPV life cycle. HPV infects the basal layer of the epithelium through micro-lesions. At this level, HPV is episomal, depending on cellular replication for encoding the necessary genes for HPV life cycle. E6 and E7 expression is needed throughout the infection, pushing cells to be mitotically active. Additionally, expression of E1, E2, E4 and E5 is crucial for viral amplification. L1 and L2 proteins are expressed in upper epithelial layers. Accidental integration or hypermethylation mechanisms lead to an unbalanced expression of E6 and E7 and consequently to the development of high-grade lesions that can progress to cancer.

64 HPVs, is able to infect the uterine cervix, oral, anal and penile mucosae [10]. The α-HPVs can be subdivided into two major subgroups, namely the low-risk HPVs (e.g. HPV6, 11, 5 and 8) which are mainly associated with development of warts and benign lesions and high-risk HPVs (e.g. HPV16, 18, 31, 33) which are considered the main drivers of high-grade lesions and cancers [13].

2. HPV-induced carcinogenesis

The integration of viral DNA into the host's genome represents a dead-end for the viral replication but is essential to drive the process of HPV-induced carcinogenesis [14]. This integration may be triggered by the genomic instability caused by the viral oncogenes E6 and E7, which increase the occurrence of double strand break in both the host's DNA and the viral genome [15]. Even though HPV integration can happen across all the human genome, it is more common in fragile chromosomal regions such as 3q28, 4q13.3, 8q24.21, 13q22.1 and 17q21 or near clusters of microRNAs [16,17]. In the HPV genomic region, the E2 ORF is usually the most affected location by the integration process [18]. The disrupted E2 HPV genomic region will also cease to act as a negative regulator of the E6 and E7 oncogenes, allowing their unbalanced expression and ultimately leading to carcinogenesis [19]. However, HPV integration is not the only factor that has been identified as a crucial step in the HPV carcinogenesis. Mechanisms of HPV

hypermethylation were found to block the access to the E2 promoter region, also leading to an unbalanced expression of the E6 and E7 viral oncogenes without E2 disruption (Fig. 2) [20,21].

2.1. Functions of HPV oncproteins

Even though all genes encoded by HPV are necessary for its life cycle and regulation, the E5, E6, and E7 ORFs of high-risk HPVs are essential for the development of HPV-positive carcinomas [22]. Therefore, it is crucial to identify and understand how these viral oncogenes and their protein products contribute to the cell alterations during carcinogenesis [23] (Table 1). In the following sections, we will discuss well-established and novel molecular targets of the E6, E7 and E5 oncogenes in impairing important regulatory pathways that are directly associated with the all known hallmarks of cancer (Figs. 3, 4, 5, 6).

2.1.1. E6 oncprotein molecular targets

The E6 oncprotein is one of the three well-established oncogenes that are associated with the malignant progression of HPV-infected cells [24]. E6 has 160 amino acids and two zinc-binding motifs, being mainly found in the cell nucleus [24]. Its interactions with multiple host cell proteins deregulate essential cellular functions and allow the development of several hallmarks of cancer (Fig. 3).

Table 1

Main functions and molecular targets of E6, E7 and E5 HPV oncoproteins ↓ Downregulation; ↑ upregulation; ✕ action blocking.

High-risk viral protein	Cellular location	Function	Main targets	Effect on target expression	References
E6	Nucleus and cytoplasm	Escaping cell death	P53 protein Procaspase 8 protein Bak protein TNR1 Fas/Fas ligand death pathway NF-κB; cIAP-2	↓ ↓ ↓ X X ↑	[26] [27,28] [29] [30] [31] [32,33]
		Deregulation of cell cycle	P300/CBP complex protein miR34a	↓ ↓	[34–36] [39,40]
		Immune system modulation	IRF3 IFN α IFN κ	↓ ↓ ↓	[43] [44] [45,46]
		Cell Immortalization	NFX1-91 Myc Sp1	↓ ↑ ↑	[48] [49] [50]
		Genomic instability	APOBEC3 XRCC1	↑ ↓	[51] [54]
		Cell invasion	Dlg SCRIB MAGI-1, MAGI-2 and MAGI-3 PAR3 Fibulin-1 miR-23b	↓ ↓ ↓ ↓ ↓ ↓	[58] [59] [61] [62] [63] [64]
			Paxillin disruption	↓ ↓	[66]
		Deregulation of cell cycle	pRB protein p107/p130 p21 p27 Claspin	↓ ↓ ↓ ↓ ↓	[70] [70] [72] [73]
		Immune system modulation	E2F6 TLR9 Cgas-STING	↓ X X	[74] [77] [78]
		Cell invasion	MMP-9	↑	[79]
		Genomic instability	Abnormal centrosome synthesis γ -Tubulin CDK2	X X ↑	[81] [82] [83]
		Deregulation of cellular energetics	Aerobic glycolysis mTORC1 GLUT-1	↑ ↑ ↑	[86] [87] [88]
		Genomic instability: epigenetic deregulation	DNMT1 DNMT3A DNMT3B E-cadherin CXCL14 CCNA1 CBP/p300 TIP60 ADA3	↑ ↑ ↑ ↓ ↓ ↓ ↓ ↓ ↓ ↓	[91–93] [94,95] [96] [97,98] [99] [101] [100]
		Inflammation promotion	IL-6 IL18	↑ ↑	[102] [103]
		Angiogenic switch	Maspin Thrombospondin-1 VEGF IL-8	↓ ↓ ↑ ↑	[105] [105] [105] [105]
		Cell Invasion	TIM-2 RECK	↓ ↓	[79] [79]
E5	Endoplasmic reticulum and Golgi apparatus	Sustaining Proliferative signalling	EGFR ATPase	↑ ↑	[109,110] [111,112]
		Escaping cell death	KGFR/FGFR2b Bax protein	↓ ↓	[113] [114]
		Cell invasion	MET	↑	[115,116]
		Immune system modulation	MHC-class I	X	[117]

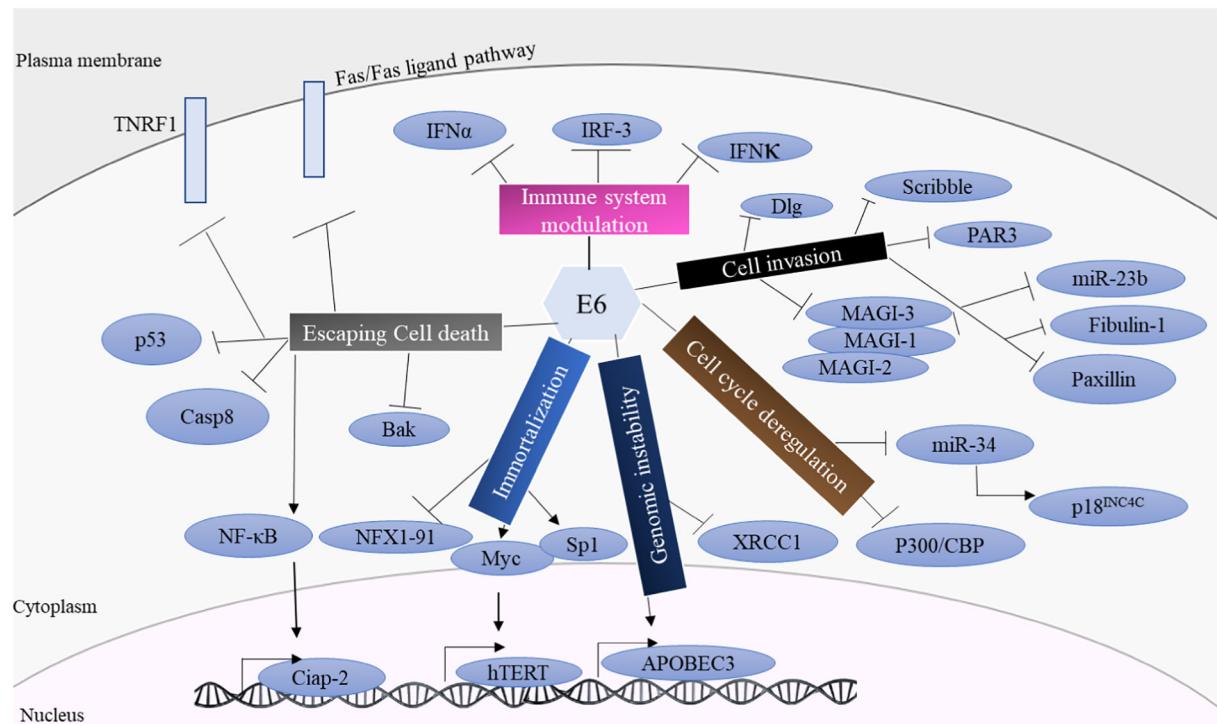


Fig. 3. Molecular targets of the high-risk E6 oncoprotein. E6 is known to interact with a diverse range of molecules that are involved in several cellular pathways namely in immune system modulation, invasion, cell cycle deregulation, genomic instability, cell immortalization and cell death, potentiating cancer development.

2.1.1.1. Escaping cell death. Escaping cell death is one of the most important hallmarks of cancer, allowing cells with genomic defects to survive and to continue proliferating [25]. The HPV oncoproteins can abrogate apoptosis, allowing cells with genomic errors to resist cell death. The most important and well-studied role of the E6 oncoprotein is the degradation of the p53 protein [26]. The E6 oncoprotein interacts with the conserved *LxxLL* consensus sequences of the Ubiquitin-protein ligase E3A (UBE3A) also known as ubiquitin ligase E6-Associated

Protein (E6-AP), that works as a connecting bridge between E6 and p53, targeting it for proteasomal degradation [26].

However, E6 can also target other molecules that are associated with the activation of different cellular death pathways. The high-risk E6 oncoprotein is able to bind and accelerate the rate of degradation of the essential pro-apoptotic protein procaspase 8 (CASP8) [27,28]. Additionally, the E6 oncoprotein can also interact with the B-cell lymphoma 2 (Bcl-2) protein family of mitochondrial regulation of apoptosis

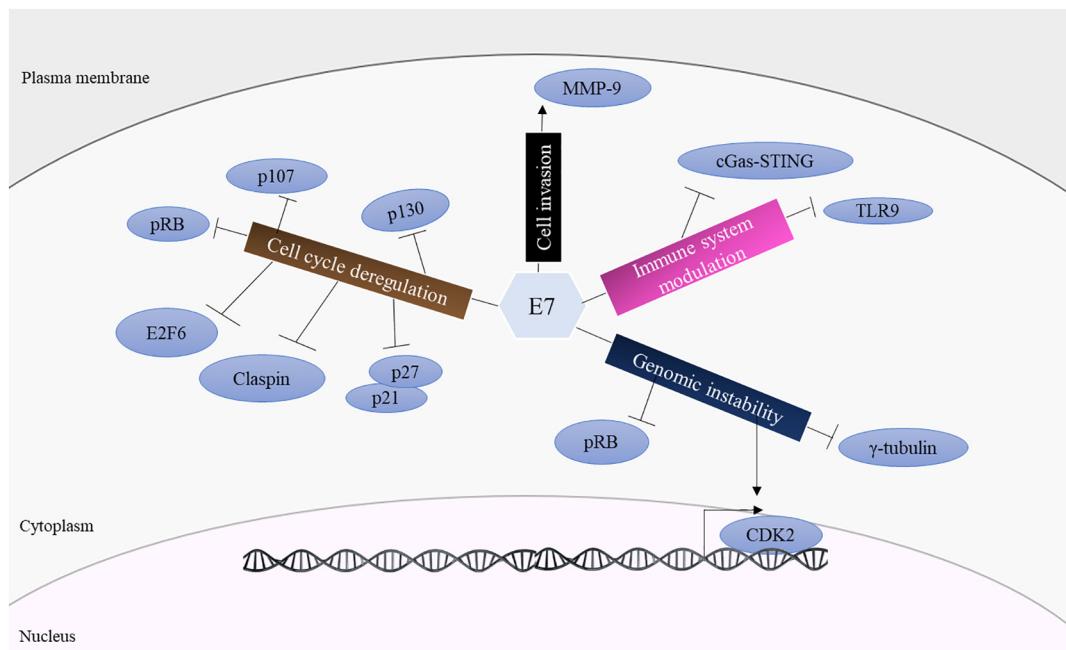


Fig. 4. Molecular targets of the high-risk E7 oncoprotein. E7 is known to interact with a diversity of molecules that are involved in several cellular pathways and therefore influence the development of cancer, such cell cycle deregulation, cell invasion, immune system modulation and genomic instability.

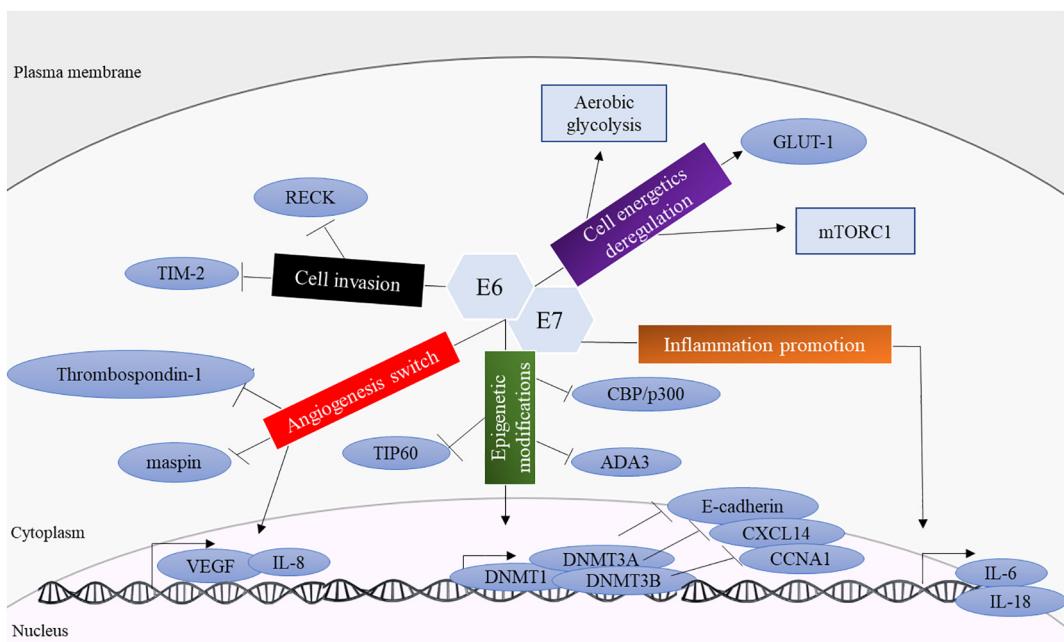


Fig. 5. Simultaneous molecular targets of the high-risk E6 and E7 oncoprotein. E6 and E7 share common targets that participate in important processes of cell invasion, cell energetics, inflammation, epigenetic modifications and angiogenesis, and this cooperation may result in a synergistic mechanism, thereby promoting malignant cell transformation.

BCL2 antagonist/killer (Bak) protein, preventing the entrance of these proteins into the mitochondria and consequently the activation of the apoptosis cascade [29]. High-risk E6 oncoprotein can also bind to the tumour necrosis factor receptor 1 (TNFR1) and inhibit TNF-mediated apoptosis [30].

Moreover, the E6 oncoprotein is also able to inhibit apoptosis through the blocking of the Fas/Fas ligand death induced pathway [31]. As previously reviewed, E6 also upregulates the protein complex nuclear factor *Kappa b* (NF- κ B), critically involved in numerous cell functions. This upregulation can lead to the transcription of anti-

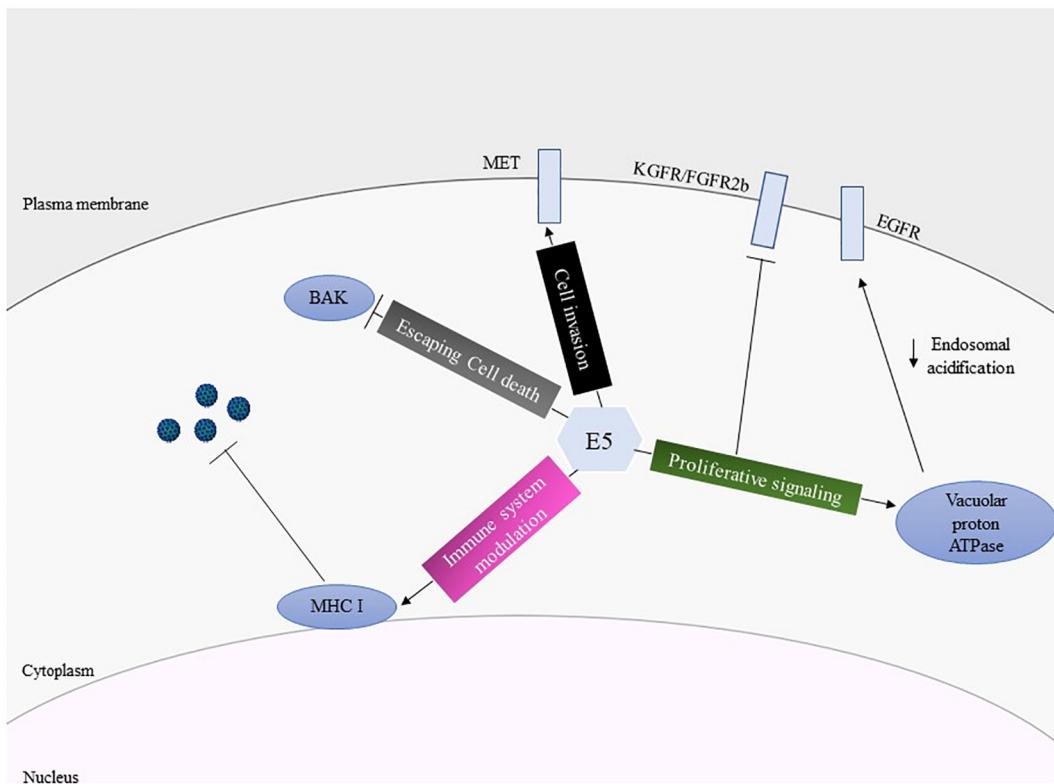


Fig. 6. Molecular targets of the high-risk E5 oncoprotein. E5 is known to interact with a diversity of molecules that are involved in several cellular pathways, such as immune system modulation, cell death and proliferation. All the presented interactions potentially influence cancer development.

apoptotic proteins like cIAP-2, preventing apoptosis [32,33].

2.1.1.2. Cell cycle deregulation. The evasion of the cell cycle checkpoints is required for deregulated cell proliferation and carcinogenesis being one of the most fundamental traits of malignant cells [25]. The E6 oncoprotein is able to deregulate the cell cycle, overcoming its checkpoints even in the presence of accumulating genomic instability [25]. E6 can inactivate the transcriptional coactivator complex p300/CBP, which is essential for regulating cell cycle progression and differentiation [34–36]. E6 also plays a vital role in the regulation of miRNAs associated with cell cycle regulation [37,38]. One notable example is the down-regulation of miR-34a through the E6-AP-E6-p53 pathway, leading to the upregulation of the p18^{Ink4c} and entrance into the S-phase [39,40].

2.1.1.3. Immune system modulation. Contrary to many viruses, HPV has a prolonged replication rate [41]. Therefore, it requires to persist for a long time in the host epithelium without being detected for which it has developed complex mechanisms of immune evasion [42]. E6 plays a role in the direct modulation of the immune system by downregulating Interferon Regulatory Factor 3 (IRF-3), a known transcription factor of Interferon β (IFN β) [43], thereby decreasing the immune response against HPV antigens. The high-risk E6 oncoprotein also targets the immune response induced by Interferon α (IFN α), by inhibiting the phosphorylation of Tyrosine Kinase 2 (TYK2) via the STAT/TYK2 pathway and therefore preventing the association between IFN α and its receptor [44]. Furthermore, the downregulation of Interferon κ (IFN κ) by E6, probably due to E6-dependent hypermethylation, reduces the signal transducer and activator of transcription 1 (STAT1), as well as expression of the Toll-like receptor-3 (TLR3), the pro-apoptotic proteins TNF superfamily member 10 (TNFSF10) and the XIAP associated factor 1 (XAF1) and p53 [45,46].

2.1.1.4. Cell immortalization. Unlimited cell proliferation is required for carcinogenesis [25] and the unbalanced expression of E6 can induce the expression of the human telomerase (hTERT), allowing for cell immortalisation [47]. hTERT overexpression occurs not only through degradation of the hTERT transcription repressor nuclear transcription factor, X-box binding 1-91 (NFX1-91) in an E6-AP dependent way, but also by the direct activation of the hTERT promoter due to the overexpression of intermediary proto-oncogene protein Myc and the transcription factor Sp1 [48–50]. The direct binding of the oncoprotein E6 to the Myc protein will recruit phosphorylated DNA polymerase II as well as promote activating epigenetic changes on the hTERT promoter region [49].

2.1.1.5. Genomic instability. The high-risk E6 oncoprotein can also independently induce genomic instability, a known hallmark of cancer, by up-regulating the apolipoprotein B mRNA editing enzyme catalytic polypeptide 3 (APOBEC3) through a mechanism still unknown [51]. This protein is commonly up-regulated in HPV-induced cervical and head and neck cancers [52] and has the primary function of replacing cytosine with an uracil residue. This mechanism is prone to errors and an up-regulation of these proteins' family eventually leads to a higher mutation rate and therefore promotes genomic instability [51,53]. Additionally, the high-risk E6 oncoprotein directly targets and inhibits the X-ray repair cross-complementing 1 (XRCC1) DNA repair protein.

This scaffold protein interacts with the DNA repair machinery participating in base excision repair and its inhibition promotes the accumulation of mutation [54].

2.1.1.6. Cell invasion. Cell invasion and metastasis is one of the most prominent hallmarks of cancer [25]. Many important functions of the high-risk E6 oncoprotein are mediated by PZD protein domains, allowing E6 to downregulate cell polarity and motility [55–57].

Several potential tumour suppressor genes, like the disc large MAGUK scaffold protein (Dgl) which regulates cell-to-cell interactions, the scribbled planar cell polarity protein (SCRIB) involved in cell polarization and differentiation, the membrane-associated guanylate kinase 1, 2 and 3 (MAGI-1, 2 and 3) that regulates cell-to-cell contact and the par-3 family cell polarity regulator (PAR3) have all been identified as targets of E6 proteolytic degradation, contributing for cell invasion [58–62]. Furthermore, the high risk E6 can also target and degrade the extracellular matrix and plasma protein Fibulin 1, a crucial cellular adhesion protein [63] and downregulate miR-23b, which is important in the regulation of the urokinase plasminogen activator gene, intimately connected with cell migration [64,65]. Additionally, E6 can interfere with paxillin, an adhesion protein, downregulating the formation of the actin fibres and consequently the integrity of the epithelium leading to invasiveness and ultimately to the metastatic processes [66].

2.1.2. E7 oncoprotein molecular targets

Among HPV oncoproteins, E7 was first discovered. This small phosphoprotein contains approximately 100 amino acid residues and three conserved regions - Conserved Region 1 (CR1), CR2 and CR3 - this last one forming a zinc finger structure [67]. The CR2 region contains an LXCXE motif that is important in the association with its targets [67] (Fig. 4).

2.1.2.1. Cell cycle deregulation. In 1989, Dyson and colleagues observed that the E7 oncoprotein targets the pRB protein inducing its ubiquitination by cullin 2-containing E3 ubiquitin ligases and consequent degradation. This allows the release of the E2F transcription factor and deregulation of cell proliferation [68]. This mechanism drives the expression of the S-phase genes cyclin A and cyclin E as well as the accumulation of p16^{INK4A}, an inhibitor cyclin-dependent kinase [69,70]. Additionally, E7 can target and degrade the "pocket proteins" p107 and p130, both of which are regulators of E2F [70,71].

Another important function of the E7 oncoprotein is the direct inactivation of essential regulators of the G1-to-S-phase transition, namely the cyclin inhibitors p21 and p27. These proteins tightly regulate cellular proliferation and their inactivation leads to unchecked cell proliferation by maintaining the cyclin dependent kinase 2 (CDK2) activity [72]. Furthermore, E7 can accelerate the turnover of claspin, a key positive regulator of the DNA damage signalling ATR-CHK1, facilitating cell proliferation even in the presence of DNA damage [73]. Last but not least, the E7 oncoprotein is able to target and inhibit the transcriptional repressor of the S-phase inducing genes, E2F6 [74].

2.1.2.2. Immune system modulation. Just like the E6 oncoprotein, E7 can disrupt interferon signalling [75] [76], and is also able to bind and inhibit the Toll-like receptor-9 (TLR9) [77]. Furthermore, the E7 oncoprotein is also able to block the cyclic GMP-AMP synthase-Stimulator of interferon genes (cGAS-STING) immune initiation pathway, a key component of the innate immune system that detects cytoplasmic DNA [78].

2.1.2.3. Cell invasion. The invasion characteristics of solid tumours often include an increased expression of matrix metalloproteinases (MMPs). MMPs have the ability to degrade components of the extracellular matrix leading to cell invasion and E7 has been shown to upregulate the expression of MMP-9 during cervical cancer progression [79].

2.1.2.4. Genomic instability. Accelerated cell proliferation promoted by high-risk HPV oncoproteins facilitates the accumulation of genetic defects like deletions, amplifications, translocations and chromosomal rearrangements [23,80]. The high-risk E7 oncoprotein plays a pivotal role in promoting genomic instability by inducing abnormal synthesis

of centrosomes, leading to aneuploidy, through pRb downregulation [81]. Furthermore, the E7 oncoprotein can also promote centrosome aberrations through pRB degradation-independent mechanisms, by degrading and removing from the mitotic spindle the crucial centrosome regulator γ -tubulin, and by increasing the activity of the CDK2, which can lead to an increased risk of genomic destabilization [82,83].

2.1.3. E6 and E7 simultaneous molecular targets

The unbalanced expression of E6 and E7 is most harmful when these two oncoproteins act cooperatively towards malignant cell transformation. Even though the E6 and E7 oncoproteins can have specific direct targets, they can also deregulate common ones, thus achieving higher efficiency in destabilising key regulatory pathways (Fig. 5).

2.1.3.1. Deregulation of cellular energetics. Carcinogenesis involves changes in energy metabolism that help sustaining growth under abnormal conditions. This alteration of cellular metabolism are among the earliest modifications observed in cancer cells and contribute to survival under conditions of poor nutrient supply [84]. Normal cells mainly rely on mitochondrial oxidative phosphorylation to produce adenosine triphosphate (ATP). However, malignant cells are usually characterized by a switch towards aerobic glycolysis, even when they are in aerobic conditions, as described by Otto Warburg [85]. Both the E6 and E7 oncoproteins contribute to the alterations [84]. The E7 oncoprotein alters the activity of the pyruvate kinase M₂-PK, upregulating the glycolytic processes [86]. On the other hand, E6 activates signalling through the mammalian target of the rapamycin complex 1 (mTORC1) and its downstream S6K effector, enhancing protein synthesis [87]. The E6 oncoprotein can also bind to the Sorting Nexin 27 (SNX27) protein, an important regulator of the endosomal transport pathway, modulating expression of the glucose transporter GLUT-1 and ultimately increasing glucose uptake by cancer cells [88].

2.1.3.2. Genomic instability: epigenetic deregulation. Another important hallmark connected with tumour proliferation is the disruption of genomic methylation patterns. In fact, HPV-driven malignancies are associated with hypermethylation and hypomethylation events affecting the expression of tumour suppressor genes and oncogenes [89,90]. Both E6 and E7 oncoproteins can promote epigenetic modifications and consequently promote genomic instability by upregulating DNA methyltransferases DNMT1, DNMT3A and DNMT3B. While E7 binds directly to DNMT1 by its CR3 zinc-finger domain, the E6 oncoprotein upregulates DNMT1 by a p53 suppression-dependent pathway [91–93].

High-risk E6 and E7-dependent hypermethylation promotes the silencing of essential tumour suppressor genes with critical regulatory functions in cell adhesion (e.g. *E-cadherin*) [94,95], inflammation (C-X-C motif chemokine ligand 14, CXCL14) [96] and cell cycle regulation (cyclin A1, CCNA1), thus promoting cancer progression, invasion and metastasis [97,98]. The E6 and E7 oncoproteins can also interact with other key post-translational modifiers namely histone acetyltransferases (HATs) and histone deacetylases (HDAC), as well as acetylation coactivators, thereby modulating transcription activity. One of the most crucial HATs inhibited by those oncoproteins is the CBP/p300 complex, involved in cell cycle progression and cell differentiation. This inactivation leads to the downregulation of crucial genes directly associated with cell cycle and apoptosis, like the tumour suppressor gene TP53 [99]. The transcriptional adaptor 3 (ADA3) acetylator coactivator is also downregulated by the HPV oncoproteins, inhibiting the CBP/p300 complexes and ultimately the transcription of p53 [100]. Additionally, the HAT Tat interactive protein 60 (TIP60) is a target for proteasomal degradation by the E6 oncoprotein, inhibiting the ability of this HAT to induce the transcription of bromodomain containing 4 (Brd4), a known E6 repressor [101].

2.1.3.3. Inflammation promotion. Both the E6 and the E7 oncoproteins can induce tumour-associated inflammation by up-regulating the expression of the pro-inflammatory cytokines Interleukin-6 (IL-6) and IL-18 [102,103]. The consequent inflammatory process leads to the up-regulation of pro-angiogenic factors, metalloproteinases and chemokines with pro-tumoral functions that support tumour progression [104].

2.1.3.4. Angiogenic switch. Malignant cells need to be able to modulate angiogenic activators and inhibitors in order to survive and to allow for tumour progression [25]. In HPV-induced cancers, the E6 and E7 oncoproteins are responsible for triggering the angiogenic switch by downregulating the angiogenic inhibitors maspin and thrombospondin-1 while upregulating the expression of Vascular Endothelial Growth Factor (VEGF) and IL-8 [105].

2.1.3.5. Cell invasion. Both the E6 and the E7 oncoproteins can also simultaneously target and disrupt important regulators of tissue homeostasis, leading to tumour invasion and to metastatic spread. One of the crucial functions of these oncoproteins is the downregulation of the Tissue Inhibitor of Metalloproteinases 2 (TIMP-2), an important regulator of MMP activity [79]. Furthermore, both E6 and E7 oncoproteins are able to target and downregulate the Reversion-Inducing Cysteine-Rich protein with Kazal motifs (RECK), a membrane protein with inhibitory functions over MMPs transcription [79].

2.1.4. E5 oncoprotein

Although the most studied transforming activities in HPV-related lesions are led by the oncoproteins E6 and E7, the E5 plays a role in carcinogenesis. E5 is a small transmembrane protein with 83 amino acids, mainly localised in the intracellular membranes of the endoplasmic reticulum and Golgi apparatus [106]. Recent studies have shown its pivotal importance in cell transformation and immune modulation, that cooperates with E7 and E6 to drive the cell's malignant progression [107,108] (Fig. 6).

2.1.4.1. Sustaining proliferative signalling. The E5 oncoprotein can be responsible for the stimulation of cancer cells' proliferation by the formation of activating complexes with growth factor receptors such as with the Epidermal Growth Factor Receptor (EGFR), leading to a continuous proliferative state [109,110]. Furthermore, in line with its location in the Golgi apparatus and in the endoplasmic reticulum, E5 also plays a vital role in cell signalling modulation by its association with the vacuolar proton ATPase, decreasing endosomal acidification [111,112]. Endosomal acidification is an important mechanism that leads to the degradation of cell surface receptors. Deregulation of endosomal acidification leads to a decreased turnover of cell surface receptors like EGFR, increasing their signalling activity [111]. Moreover, the E5 oncoprotein can also target and downregulate the keratinocyte growth factor receptor/fibroblast growth factor receptor 2b (KGFR/FGFR2b) signalling, consequently decreasing the autophagy process [113].

2.1.4.2. Escaping cell death. The E5 oncoprotein like the E6 also plays a role in decreasing cell death and therefore promoting the accumulation of cells with abnormal DNA genetic mutations and consequently promoting the malignancy process. E5 is able to target and inhibit apoptosis by increasing the ubiquitination and proteasomal degradation of the pro-apoptotic protein Bax [114].

2.1.4.3. Cell invasion. Most studies regarding HPV carcinogenesis and invasiveness have been focused on the action of the high-risk E6 and E7 oncoprotein, however, recent studies have shown that the E5 oncoprotein can have a pivotal importance in the HPV-induced cancers, especially in metastatic process by up-regulating the

expression of MET, an hepatocyte growth factor receptor (HGFR) [115,116]. This overexpression induced by the action of the E5 oncoprotein leads to a more severe progression of lesions and a lower survival of patients [115,116].

2.1.4.4. Immune system modulation. One of the most important roles of the E5 oncoprotein is its modulation of the immune system. E5 can interact with the Major-Histocompatibility Complex class I (MHC-class I). The E5 oncoprotein promotes the retention of the MHC class I in the Golgi apparatus, inhibiting its transportation to the cell surface, a fact that leads to a decreased ability of the complex to present the viral antigens to the T-cells [117]. Additionally, the E5 antigens are poorly recognised by CD8⁺ T cells, facilitating the immune evasion of HPV-transformed cells [118]. Overall, the E5 oncoprotein seems to play an additive role in carcinogenesis together with E6 and E7, rather than having a major transformation role by itself. However, more studies showing the malignant potential of the E5 oncoproteins are now being uncovered taking it from the shadow of the E6 and E7 oncoproteins.

3. E6, E7 and E5 as therapeutic targets?

Genome editing tools have accelerated cancer research, especially those applications involving the Clustered Regularly Interspaced Short Palindromic Repeats-Associated Protein 9 (CRISPR-Cas9) [119,120]. Since the HPV oncoproteins are the main drivers of cellular malignancy, it is tempting to use CRISPR technology to disrupt the *E6* and *E7* genes, restoring the function of the previously impaired regulatory pathways. One of the first successful attempts to target the action of E6 and E7 oncoproteins was achieved by Shuai Zhen and colleagues, who employed CRISPR-Cas9 technology to manipulate HPV16-positive SiHa cells. The disruption of the *E6* and *E7* genes restored the expression of tumour suppressor proteins p53 and p21 activating p53-dependent apoptosis mechanisms and arresting the cell cycle. Furthermore, CRISPR-Cas9 edited cells were xenografted into nude mice and showed reduced tumour growth compared with untreated cells [121]. Lan Yu and colleagues also used CRISPR-Cas9 to disrupt the *E6* gene not only in SiHa but also in CaSki cells. These authors showed the restoration of the p53 protein alongside with the downregulation of E6 [122]. These promising results showed how genome editing tools, like the CRISPR-Cas9, can be used to target HPV oncoproteins and interfere with the malignant phenotype of HPV-transformed cells. No studies using CRISPR-Cas9 to disrupt the E5 oncoprotein have been attempted so far.

4. Conclusion

HPV-related cancers are still a major worldwide health concern and it is crucial to fully understand the oncogenic mechanisms involved in their development. Advances in mass spectrometry technology allowed the identification of several molecular targets of the E6, E7 and E5 oncoproteins. These viral proteins have the remarkable capability to impair multiple key regulatory pathways and elicit all the known hallmarks associated with cancer. While conventional therapeutic approaches such as surgery, chemotherapy, and radiotherapy remain the mainstay of treatment for cancer patients, tumour resistance and recurrence are frequently observed, decreasing the survival and quality of life of patients. The possibility of genome-editing tools, to edit-out viral oncogenes and treat HPV-related malignancies, needs to be further supported by *in vivo* studies demonstrating efficacy in relevant models of cancer.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgments

We acknowledge the Portuguese League Against Cancer (Liga Portuguesa Contra o Cancro—Núcleo Regional do Norte), and the Research Center of the Portuguese Institute of Oncology of Porto (CIPOP 37-2016) for the support.

References

- [1] B. Serrano, M. Brotons, F.X. Bosch, L. Bruni, Epidemiology and burden of HPV-related disease, *Best Pract. Res. Clin. Obstet. Gynaecol.* 47 (2017) 14–26.
- [2] H. zur Hausen, Condylomata acuminata and human genital cancer, *Cancer Res.* 36 (2 pt 2) (1976) 794.
- [3] World HPV Information Center, Human papillomavirus and related diseases report, ICO HPV Inf. Cent. Rep. (July) (2017) 1–334.
- [4] K. Wakeham, K. Kavanagh, The burden of HPV-associated anogenital cancers, *Curr. Oncol. Rep.* 16 (9) (2014) 402.
- [5] C. de Martel, M. Plummer, J. Vignat, S. Franceschi, Worldwide burden of cancer attributable to HPV by site, country and HPV type, *Int. J. Cancer* 141 (4) (2017) 664–670.
- [6] S. Vaccarella, J. Lortet-Tieulent, M. Plummer, S. Franceschi, F. Bray, Worldwide trends in cervical cancer incidence: impact of screening against changes in disease risk factors, *Eur. J. Cancer* 49 (15) (2013) 3262–3273.
- [7] A.K. Chaturvedi, E.A. Engels, R.M. Pfeiffer, et al., Human papillomavirus and rising oropharyngeal cancer incidence in the United States, *J. Clin. Oncol.* 29 (32) (2011) 4294–4301.
- [8] A. Auluck, G. Hislop, C. Bajdik, C. Poh, L. Zhang, M. Rosin, Trends in oropharyngeal and oral cavity cancer incidence of human papillomavirus (HPV)-related and HPV-unrelated sites in a multicultural population: the British Columbia experience, *Cancer* 116 (11) (2010) 2635–2644.
- [9] J. Doorbar, Nagayasu Egawa, Heather Griffin, CK, IM, Human papillomavirus molecular biology and disease association, *Rev. Med. Virol.* 25 (1) (2016) 2–23.
- [10] N. Egawa, K. Egawa, H. Griffin, J. Doorbar, Human papillomaviruses; epithelial tropisms, and the development of neoplasia, *Viruses* 7 (7) (2015) 3863–3890.
- [11] M.E. Harden, K. Munger, Human papillomavirus molecular biology, *Mutat. Res.* 772 (2016) 3–12.
- [12] S.V. Graham, Keratinocyte differentiation-dependent human papillomavirus gene regulation, *Viruses* 9 (9) (2017).
- [13] K. Van Doorslaer, Evolution of the papillomaviridae, *Virology* 445 (1–2) (2013) 11–20.
- [14] S.R. Georgescu, C.I. Mitran, M.I. Mitran, et al., New insights in the pathogenesis of HPV infection and the associated carcinogenic processes: the role of chronic inflammation and oxidative stress, *J. Immunol. Res.* 2018 (2018) 1–10.
- [15] M.A. Oyervides-Muñoz, A.A. Pérez-Maya, H.F. Rodríguez-Gutiérrez, et al., Understanding the HPV integration and its progression to cervical cancer, *Infect. Genet. Evol.* 61 (February) (2018) 134–144.
- [16] G. Gao, S.H. Johnson, G. Vasmatzis, et al., Common fragile sites (CFS) and extremely large CFS genes are targets for human papillomavirus integrations and chromosome rearrangements in oropharyngeal squamous cell carcinoma, *Genes Chromosom. Cancer* 56 (1) (2017) 59–74.
- [17] M.K. Jang, K. Shen, A.A. McBride, Papillomavirus genomes associate with BRD4 to replicate at fragile sites in the host genome, *PLoS Pathog.* 10 (5) (2014).
- [18] C.A. Moody, L.A. Laimins, Human papillomavirus oncoproteins: pathways to transformation, *Nat. Rev. Cancer* 10 (8) (2010) 550–560.
- [19] S.V. Graham, The human papillomavirus replication cycle, and its links to cancer progression: a comprehensive review, *Clin. Sci.* 131 (17) (2017) 2201–2221.
- [20] V. Simanaviciene, V. Popendikyte, Z. Gudleviciene, A. Zvirbliene, Different DNA methylation pattern of HPV16, HPV18 and HPV51 genomes in asymptomatic HPV infection as compared to cervical neoplasia, *Virology* 484 (2015) 227–233.
- [21] S. Sen, P. Mandal, A. Bhattacharya, et al., Impact of viral and host DNA methylations on HPV16-related cervical cancer pathogenesis, *Tumour Biol.* 39 (5) (2017) 1010428317699799.
- [22] R.B.S. Roden, P.L. Stern, Opportunities and challenges for human papillomavirus vaccination in cancer, *Nat. Rev. Cancer* 18 (4) (2018).
- [23] S. Mittal, L. Banks, Molecular mechanisms underlying human papillomavirus E6 and E7 oncoprotein-induced cell transformation, *Mutat. Res. Mutat. Res.* 772 (2016) 23–35.
- [24] H.L. Howie, R.A. Katzenellenbogen, D.A. Galloway, Papillomavirus E6 proteins, *Virology* 384 (2) (2008) 324–334.
- [25] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, *Cell* 144 (2011).
- [26] D. Martinez-zapien, F.X. Ruiz, J. Poirson, et al., Structure of the E6/E6AP/p53 complex required for HPV-mediated degradation of p53, *Nature* 529 (7587) (2016) 541–545.
- [27] M. Filippova, M.M. Johnson, M. Bautista, et al., The large and small isoforms of human papillomavirus type 16 E6 bind to and differentially affect procaspase 8 stability and activity, *J. Virol.* 81 (8) (2007) 4116–4129.
- [28] T.O. Garnett, M. Filippova, P.J. Duerksen-Hughes, Accelerated degradation of FADD and procaspase 8 in cells expressing human papilloma virus 16 E6 impairs TRAIL-mediated apoptosis, *Cell Death Differ.* 13 (11) (2006) 1915–1926.
- [29] M. Thomas, L. Banks, Human papillomavirus (HPV) E6 interactions with Bak are conserved amongst E6 proteins from high and low risk HPV types, *J. Gen. Virol.* 80 (1999) 1513–1517.

- [30] M. Filippova, H. Song, J.L. Connolly, T.S. Dermody, P.J. Duerksen-Hughes, The human papillomavirus 16 E6 protein binds to tumor necrosis factor (TNF) R1 and protects cells from TNF-induced apoptosis, *J. Biol. Chem.* 277 (24) (2002) 21730–21739.
- [31] M. Filippova, L. Parkhurst, P.J. Duerksen-Hughes, The human papillomavirus 16 E6 protein binds to Fas-associated death domain and protects cells from Fas-triggered apoptosis, *J. Biol. Chem.* 279 (24) (2004) 25729–25744.
- [32] M.A. James, J.H. Lee, A.J. Klingelhutz, Human papillomavirus type 16 E6 activates NF-κappaB, induces cIAP-2 expression, and protects against apoptosis in a PDZ binding motif-dependent manner, *J. Virol.* 80 (11) (2006) 5301–5307.
- [33] R.M.G. Da Costa, M.M.S.M. Bastos, R. Medeiros, P.A. Oliveira, The NFκB signaling pathway in papillomavirus-induced lesions: friend or foe? *Anticancer Res.* 36 (5) (2016) 2073–2083.
- [34] D. Patel, S.M. Huang, L.A. Baglia, D.J. McCance, The E6 protein of human papillomavirus type 16 binds to and inhibits co-activation by CBP and p300, *EMBO J.* 18 (18) (1999) 5061–5072.
- [35] E.A. White, R.E. Kramer, M.J.A. Tan, S.D. Hayes, J.W. Harper, P.M. Howley, Comprehensive analysis of host cellular interactions with human papillomavirus E6 proteins identifies new E6 binding partners and reflects viral diversity, *J. Virol.* 86 (24) (2012) 13174–13186.
- [36] X. Xie, L. Piao, B.N. Bullock, et al., Targeting HPV16 E6-p300 interaction re-activates p53 and inhibits the tumorigenicity of HPV-positive head and neck squamous cell carcinoma, *Oncogene* 33 (8) (2014) 1037–1046.
- [37] M.V. Chiantore, G. Mangino, M. Iuliano, et al., Human papillomavirus E6 and E7 oncoproteins affect the expression of cancer-related microRNAs: additional evidence in HPV-induced tumorigenesis, *J. Cancer Res. Clin. Oncol.* 142 (8) (2016) 1751–1763.
- [38] J. Ribeiro, J. Marinho-Dias, P. Monteiro, et al., miR-34a and miR-125b Expression in HPV Infection and Cervical Cancer Development, *Biomed. Res. Int.* 2015 (2015) 304584.
- [39] X. Wang, C. Meyers, M. Guo, Z.-M. Zheng, Up-regulation of p18Ink4c expression by oncogenic HPV E6 via p53-miR-34a pathway, *Int. J. Cancer* 129 (6) (2011) 1362–1372.
- [40] Y. Zhu, Y. Han, T. Tian, et al., MiR-21-5p, miR-34a, and human telomerase RNA component as surrogate markers for cervical cancer progression, *Pathol. Res. Pract.* 214 (3) (2018) 374–379.
- [41] T. Reinson, L. Henno, M. Toots, M. Ustav, M. Ustav, The cell cycle timing of human papillomavirus DNA replication, *PLoS One* 10 (7) (2015) 1–16.
- [42] A. Steinbach, A.B. Riemer, Immune evasion mechanisms of human papillomavirus: an update, *Int. J. Cancer* (2017) 1–11.
- [43] M. Shah, M.A. Anwar, S. Park, S.S. Jafri, S. Choi, In silico mechanistic analysis of IRF3 inactivation and high-risk HPV E6 species-dependent drug response, *Sci. Rep.* 5 (August 2014) (2015) 1–14.
- [44] S. Li, S. Labrecque, M.C. Gauzzi, et al., The human papilloma virus (HPV)-18 E6 oncoprotein physically associates with Tyk2 and impairs Jak-STAT activation by interferon-alpha, *Oncogene* 18 (42) (1999) 5727–5737.
- [45] B. Rincon-Orozco, G. Halec, S. Rosenberger, et al., Epigenetic silencing of interferon-kappa in human papillomavirus type 16-positive cells, *Cancer Res.* 69 (22) (2009) 8718–8725.
- [46] J. Reiser, J. Hurst, M. Voges, et al., High-risk human papillomaviruses repress constitutive kappa interferon transcription via E6 to prevent pathogen recognition receptor and antiviral-gene expression, *J. Virol.* 85 (21) (2011) 11372–11380.
- [47] A. Pańczyszyn, E. Boniewska-Bernacka, G. Głab, Telomeres and telomerase during human papillomavirus-induced carcinogenesis, *Mol. Diagn. Ther.* 22 (4) (2018) 421–430.
- [48] L. Gewin, H. Myers, T. Kiyono, D.A. Galloway, Identification of a novel telomerase repressor that interacts with the human papillomavirus type-16 E6/E6-AP complex, *Genes Dev.* 18 (18) (2004) 2269–2282.
- [49] Y. Zhang, A. Dakic, R. Chen, Y. Dai, R. Schlegel, X. Liu, Direct HPV E6/Myc interactions induce histone modifications, Pol II phosphorylation, and hTERT promoter activation, *Oncotarget* 8 (56) (2017) 96323–96339.
- [50] S. Oh, S. Kyo, L. Laimins, Telomerase activation by human papillomavirus type 16 E6 protein: induction of human telomerase reverse transcriptase expression through Myc and telomerase activation by human papillomavirus type 16 E6 protein : induction of human telomerase reverse Tran, *J. Virol.* 75 (12) (2001) 5559.
- [51] V.C. Vieira, B. Leonard, E.A. White, et al., Human papillomavirus E6 triggers upregulation of the antiviral and cancer genomic DNA deaminase APOBEC3B, *MBio* 5 (6) (2014) 1–8.
- [52] S. Henderson, A. Chakravarthy, X. Su, C. Boshoff, T.R. Fenton, APOBEC-mediated cytosine deamination links PIK3CA helical domain mutations to human papillomavirus-driven tumor development, *Cell Rep.* 7 (6) (2014) 1833–1841.
- [53] C.J. Warren, J.A. Westrich, K. Van Doorslaer, D. Pyeon, Roles of APOBEC3A and APOBEC3B in human papillomavirus infection and disease progression, *Viruses* 9 (8) (2017) 1–20.
- [54] T. Iftner, M. Elbel, B. Schopp, et al., Interference of papillomavirus E6 protein with single-strand break repair by interaction with XRCC1, *EMBO J.* 21 (17) (2002) 4741–4748.
- [55] C.P. Delury, E.K. Marsh, C.D. James, et al., The role of protein kinase A regulation of the E6 PDZ-binding domain during the differentiation-dependent life cycle of human papillomavirus type 18, *J. Virol.* 87 (17) (2013) 9463–9472.
- [56] C. James, S. Roberts, Viral interactions with PDZ domain-containing proteins—an oncogenic trait? *Pathogens* 5 (1) (2016) 8.
- [57] Y. Yoshimatsu, T. Nakahara, K. Tanaka, et al., Roles of the PDZ-binding motif of HPV 16 E6 protein in oncogenic transformation of human cervical keratinocytes, *Cancer Sci.* 108 (7) (2017) 1303–1309.
- [58] S.S. Lee, R.S. Weiss, R.T. Javier, Binding of human virus oncoproteins to hDlg/SAP97, a mammalian homolog of the *Drosophila* discs large tumor suppressor protein, *Proc. Natl. Acad. Sci. U. S. A.* 94 (13) (1997) 6670–6675.
- [59] C. Kranjec, V. Tomaic, P. Massimi, L. Nicolaides, J. Doorbar, L. Banks, The high-risk HPV E6 target scribble (hScrib) is required for HPV E6 expression in cervical tumour-derived cell lines, *Papillomavirus Res.* (Amsterdam, Netherlands) 2 (2016) 70–77.
- [60] S. Nakagawa, J.M. Huibregtse, Human scribble (Vartul) is targeted for ubiquitin-mediated degradation by the high-risk papillomavirus E6 proteins and the E6AP ubiquitin-protein ligase, *Mol. Cell. Biol.* 20 (21) (2000) 8244–8253.
- [61] M. Thomas, R. Laura, K. Hepner, et al., Oncogenic human papillomavirus E6 proteins target the MAGI-2 and MAGI-3 proteins for degradation, *Oncogene* 21 (33) (2002) 5088–5096.
- [62] F. Facciuto, M. Bugnon, F. Marziali, et al., Human papillomavirus (HPV)-18 E6 oncoprotein interferes with the epithelial cell polarity Par3 protein, *Mol. Oncol.* 8 (3) (2014) 533–543.
- [63] M. Du, X. Fan, E. Hong, J.J. Chen, Interaction of oncogenic papillomavirus E6 proteins with fibulin-1, *Biochem. Biophys. Res. Commun.* 296 (4) (2002) 962–969.
- [64] C.L. Au Yeung, T.Y. Tsang, P.L. Yau, T.T. Kwok, Human papillomavirus type 16 E6 induces cervical cancer cell migration through the p53/microRNA-23b/urokinase-type plasminogen activator pathway, *Oncogene* 30 (21) (2011) 2401–2410.
- [65] C.L.A. Yeung, T.Y. Tsang, P.L. Yau, T.T. Kwok, Human papillomavirus type 16 E6 suppresses microRNA-23b expression in human cervical cancer cells through DNA methylation of the host gene C9orf3, *Oncotarget* 8 (7) (2017) 12158–12173.
- [66] D.-W. Wu, C.-Y. Chuang, W.-L. Lin, W.-W. Sung, Y.-W. Cheng, H. Lee, Paxillin promotes tumor progression and predicts survival and relapse in oral cavity squamous cell carcinoma by microRNA-218 targeting, *Carcinogenesis* 35 (8) (2014) 1823–1829.
- [67] A. Roman, K. Munger, The papillomavirus E7 proteins, *Virology* 29445 (0) (2013) 138–168.
- [68] S.G. Hwang, D. Lee, J. Kim, T. Seo, J. Choe, Human papillomavirus type 16 E7 binds to E2F1 and activates E2F1-driven transcription in a retinoblastoma protein-independent manner, *J. Biol. Chem.* 277 (4) (2002) 2923–2930.
- [69] Z. Hu, L. Yu, D. Zhu, et al., Disruption of HPV16-E7 by CRISPR/Cas system induces apoptosis and growth inhibition in HPV16 positive human cervical cancer cells, *Biomed. Res. Int.* 2014 (2014) 9 (Article ID 612823).
- [70] S.L. Gonzalez, M. Strelmau, X. He, J.R. Basile, K. Munger, Degradation of the retinoblastoma tumor suppressor by the human papillomavirus type 16 E7 oncoprotein is important for functional inactivation and is separable from proteasomal degradation of E7, *J. Virol.* 75 (16) (2001) 7583–7591.
- [71] J.A. DeCaprio, Human papillomavirus type 16 E7 perturbs DREAM to promote cellular proliferation and mitotic gene expression, *Oncogene* 33 (2014) 4036.
- [72] M. Fischer, S. Uxa, C. Stanko, T.M. Magin, K. Engelstad, Human papilloma virus E7 oncoprotein abrogates the p53-p21-DREAM pathway, *Sci. Rep.* 7 (1) (2017) 1–11.
- [73] N. Spardy, K. Covella, E. Cha, et al., HPV-16 E7 attenuates DNA damage checkpoint control by increasing the proteolytic turnover of claspin, *Cancer Res.* 69 (17) (2009) 7022–7029.
- [74] M.E. McLaughlin-Drubin, K.-W. Huh, K. Munger, Human papillomavirus type 16 E7 oncoprotein associates with E2F6, *J. Virol.* 82 (17) (2008) 8695–8705.
- [75] W.K. Songock, S. Kim, J.M. Bodily, L. State, The human papillomavirus E7 oncoprotein as a regulator of transcription, *Virus Res.* 231 (2017) 56–75.
- [76] L.B. Ivashkiv, L.T. Donlin, Regulation of type I interferon responses, *Nat. Rev. Immunol.* 14 (1) (2014) 36–49.
- [77] U.A. Hasan, C. Zannetti, P. Parroche, et al., The human papillomavirus type 16 E7 oncoprotein induces a transcriptional repressor complex on the Toll-like receptor 9 promoter, *J. Exp. Med.* 210 (7) (2013) 1369–1387.
- [78] A. Lau, E.E. Gray, R.L. Brunette, D.B. Stetson, DNA tumor virus oncogenes antagonize the cGAS-STING DNA-sensing pathway, *Science* 350 (6260) (2015) 568–571 (80–).
- [79] L.B. da Silva Cardeal, E. Boccardo, L. Termini, et al., HPV16 oncoproteins induce MMPs/RECK-TIMP-2 imbalance in primary keratinocytes: possible implications in cervical carcinogenesis, *PLoS One* 7 (3) (2012) 1–9.
- [80] K. Akagi, J. Li, T.R. Broutian, et al., Genome-wide analysis of HPV integration in human cancers reveals recurrent, focal genomic instability, *Genome Res.* 24 (2) (2014) 185–199.
- [81] S. Duensing, A. Duensing, C.P. Crum, K. Munger, Human papillomavirus type 16 E7 oncoprotein-induced abnormal centrosome synthesis is an early event in the evolving malignant phenotype, *Cancer Res.* 61 (6) (2001) 2356–2360.
- [82] C.L. Nguyen, C. Eichwald, M.L. Nibert, K. Münger, K. Munger, Human papillomavirus type 16 E7 oncoprotein associates with the centrosomal component gamma-tubulin, *J. Virol.* 81 (24) (2007) 13533–13543.
- [83] W. He, D. Staples, C. Smith, C. Fisher, Direct activation of cyclin-dependent kinase 2 by human papillomavirus E7, *J. Virol.* 77 (19) (2003) 10569–10574.
- [84] I. Martínez-Ramírez, A. Carrillo-García, A. Contreras-Paredes, E. Ortiz-Sánchez, A. Cruz-Gregorio, M. Lizano, Regulation of cellular metabolism by high-risk human papillomaviruses, *Int. J. Mol. Sci.* 19 (7) (2018).
- [85] W.H. Koppenol, P.L. Bounds, C.V. Dang, Otto Warburg's contributions to current concepts of cancer metabolism, *Nat. Rev. Cancer* 11 (5) (2011) 325–337.
- [86] W. Zwerschke, S. Mazurek, P. Massimi, L. Banks, E. Eigenbrodt, P. Jansen-Dürr, Modulation of type M2 pyruvate kinase activity by the human papillomavirus type 16 E7 oncoprotein, *Proc. Natl. Acad. Sci. U. S. A.* 96 (4) (1999) 1291–1296.
- [87] J.M. Spangle, K. Munger, The human papillomavirus type 16 E6 oncoprotein activates mTORC1 signaling and increases protein synthesis, *J. Virol.* 84 (18) (2010) 9398–9407.
- [88] K. Ganti, P. Massimi, J. Manzo-Merino, et al., Interaction of the human papillomavirus E6 oncoprotein with sorting nexin 27 modulates endocytic cargo

- transport pathways, *PLoS Pathog.* 12 (9) (2016) 1–22.
- [89] M. Lechner, T. Fenton, J. West, et al., Identification and functional validation of HPV-mediated hypermethylation in head and neck squamous cell carcinoma, *Genome Med.* 5 (2) (2013) 1–16.
- [90] R.D.M. Steenbergen, M. Ongenaert, S. Snellenberg, et al., Methylation-specific digital karyotyping of HPV16E6E7-expressing human keratinocytes identifies novel methylation events in cervical carcinogenesis, *J. Pathol.* 231 (1) (2013) 53–62.
- [91] C.L. Au Yeung, W.P. Tsang, T.Y. Tsang, N.N. Co, P.L. Yau, T.T. Kwok, HPV-16 E6 upregulation of DNMT1 through repression of tumor suppressor p53, *Oncol. Rep.* 24 (6) (2010) 1599–1604.
- [92] C.H. Hsu, K.L. Peng, H.C. Jhang, et al., The HPV E6 oncoprotein targets histone methyltransferases for modulating specific gene transcription, *Oncogene* 31 (18) (2012) 2335–2349.
- [93] W.A. Burgers, L. Blanchon, S. Pradhan, Y. De Launoit, T. Kouzarides, F. Fuks, Viral oncoproteins target the DNA methyltransferases, *Oncogene* 26 (11) (2007) 1650–1655.
- [94] Z.J. D'Costa, C. Jolly, E.J. Androphy, A. Mercer, C.M. Matthews, M.H. Hibma, Transcriptional repression of E-cadherin by human papillomavirus type 16 E6, *PLoS One* 7 (11) (2012).
- [95] J. Laurson, S. Khan, R. Chung, K. Cross, K. Raj, Epigenetic repression of E-cadherin by human papillomavirus 16 E7 protein, *Carcinogenesis* 31 (5) (2010) 918–926.
- [96] L. Cicchini, J.A. Westrich, T. Xu, et al., Suppression of antitumor immune responses by human papillomavirus through epigenetic downregulation of CXCL14, *MBio* 7 (3) (2016).
- [97] K. Chalertpet, W. Pakdeechaidan, V. Patel, A. Mutirangura, P. Yanatatsaneejit, Human papillomavirus type 16 E7 oncoprotein mediates CCNA1 promoter methylation, *Cancer Sci.* 106 (10) (2015) 1333–1340.
- [98] S. Chujan, N. Kitkumthorn, S. Siriangkul, A. Mutirangura, CCNA1 promoter methylation: a potential marker for grading Papanicolaou smear cervical squamous intraepithelial lesions, *Asian Pac. J. Cancer Prev.* 15 (18) (2014) 7971–7975.
- [99] M.C. Thomas, C.M. Chiang, E6 oncoprotein represses p53-dependent gene activation via inhibition of protein acetylation independently of inducing p53 degradation, *Mol. Cell* 17 (2) (2005) 251–264.
- [100] A. Kumar, Y. Zhao, G. Meng, et al., Human papillomavirus oncoprotein E6 inactivates the transcriptional coactivator human ADA3, *Mol. Cell. Biol.* 22 (16) (2002) 5801–5812.
- [101] S. Jha, S. Vande Pol, N.S. Banerjee, A.B. Dutta, L.T. Chow, A. Dutta, Destabilization of TIP60 by human papillomavirus E6 results in attenuation of TIP60 dependent transcriptional regulation and apoptotic pathway, *Mol. Cell* 38 (5) (2010) 700–711.
- [102] C. Ren, X. Cheng, B. Lu, G. Yang, Activation of interleukin-6/signal transducer and activator of transcription 3 by human papillomavirus early proteins 6 induces fibroblast senescence to promote cervical tumourigenesis through autocrine and paracrine pathways in tumour microenvironment, *Eur. J. Cancer* 49 (18) (2013) 3889–3899.
- [103] K.H. Richards, R. Doble, C.W. Wasson, et al., Human papillomavirus E7 oncoprotein increases production of the anti-inflammatory Interleukin-18 binding protein in keratinocytes, *J. Virol.* 88 (8) (2014) 4173–4179.
- [104] L.S. Hammes, R. Rao, P. Naud, et al., Macrophages, inflammation and risk of cervical intraepithelial neoplasia (CIN) progression — clinicopathological correlation, *Gynecol. Oncol.* 105 (2007) 157–165.
- [105] E. Toussaint-Smith, D.B. Donner, A. Roman, Expression of human papillomavirus type 16 E6 and E7 oncoproteins in primary foreskin keratinocytes is sufficient to alter the expression of angiogenic factors, *Oncogene* 23 (17) (2004) 2988–2995.
- [106] A. Venuti, F. Paolini, L. Nasir, et al., Papillomavirus E5: the smallest oncoprotein with many functions, *Mol. Cancer* 10 (140) (2011) 1–18.
- [107] J.P. Maufort, A. Shai, H. Pitot, P.F. Lambert, A role for HPV 16 E5 in cervical, *Carcinogenesis* 70 (7) (2011) 2924–2931.
- [108] A.C. De Freitas, T.H.A. De Oliveira, M.R. Barros, A. Venuti, HrHPV E5 oncoprotein: immune evasion and related immunotherapies, *J. Exp. Clin. Cancer Res.* 36 (1) (2017) 1–15.
- [109] D. Pim, M. Collins, L. Banks, Human papillomavirus type 16 E5 gene stimulates the transforming activity of the epidermal growth factor receptor, *Oncogene* 7 (1) (1992) 27–32.
- [110] C.W. Wasson, E.L. Morgan, M. Müller, et al., Human papillomavirus type 18 E5 oncogene supports cell cycle progression and impairs epithelial differentiation by modulating growth factor receptor signalling during the virus life cycle, *Oncotarget* 8 (61) (2017) 103581–103600.
- [111] S.W. Straight, B. Herman, McCance DJ, The E5 oncoprotein of human papillomavirus type 16 inhibits the acidification of endosomes in human keratinocytes, *J. Virol.* 69 (5) (1995) 3185–3192.
- [112] D.R. Mahato, W.B. Fischer, Specification of binding modes between a transmembrane peptide mimic of ATP6VOC and polytopic E5 of human papillomavirus-16, *J. Biomol. Struct. Dyn.* 36 (10) (2018) 2618–2627.
- [113] F. Belleudi, L. Leone, V. Purpura, F. Cannella, C. Scrofani, M.R. Torrisi, HPV16 E5 affects the KGFR/FGFR2b-mediated epithelial growth through alteration of the receptor expression, signaling and endocytic traffic, *Oncogene* 30 (50) (2011) 4963–4976.
- [114] J.M. Oh, S.H. Kim, E.A. Cho, Y.S. Song, W.H. Kim, Y.S. Juhnn, Human papillomavirus type 16 E5 protein inhibits hydrogen peroxide-induced apoptosis by stimulating ubiquitin-proteasome-mediated degradation of Bax in human cervical cancer cells, *Carcinogenesis* 31 (3) (2010) 402–410.
- [115] M.L. Scott, D.T. Coleman, K.C. Kelly, et al., Human papillomavirus type 16 E5-mediated upregulation of Met in human keratinocytes, *Virology* 519 (March) (2018) 1–11.
- [116] N. Hemmat, H.B. Baghi, Human papillomavirus E5 protein, the undercover culprit of tumorigenesis, *Infect. Agent. Cancer* 3 (13:31) (2018) 4–5.
- [117] G.H. Ashrafi, M. Haghshenas, B. Marchetti, M.S. Campo, E5 protein of human papillomavirus 16 downregulates HLA class I and interacts with the heavy chain via its first hydrophobic domain, *Int. J. Cancer* 119 (9) (2006) 2105–2112.
- [118] M.S. Campo, S.V. Graham, M.S. Cortese, et al., HPV-16 E5 down-regulates expression of surface HLA class I and reduces recognition by CD8 T cells, *Virology* 407 (1) (2010) 137–142.
- [119] L.A. Marraffini, CRISPR-Cas immunity against phages: its effects on the evolution and survival of bacterial pathogens, *PLoS Pathog.* 9 (12) (2013) 1–4.
- [120] D. Estêvão, N. Rios Costa, R.G. da Costa, R. Medeiros, CRISPR-Cas9 therapies in experimental mouse models of cancer, *Future Oncol.* 14 (20) (2018) 2083–2095.
- [121] S. Zhen, J.-J. Lu, L.-J. Wang, et al., In vitro and in vivo synergistic therapeutic effect of cisplatin with human papillomavirus16 E6/E7 CRISPR/Cas9 on cervical cancer cell line, *Transl. Oncol.* 9 (6) (2016) 498–504.
- [122] L. Yu, X. Wang, D. Zhu, et al., Disruption of human papillomavirus 16 E6 gene by clustered regularly interspaced short palindromic repeat/Cas system in human cervical cancer cells, *Onco. Targets Ther.* 8 (2014) 37–44.