

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

7,000

Open access books available

187,000

International authors and editors

205M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Human Papillomaviruses Oncoproteins

Gabriela Anton, Adriana Plesa, Coralia Bleotu, Anca Botezatu, Mariana Anton, Lorelei Irina Brasoveanu and Mihai Stoian

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55342>

1. Introduction

One of the most common sexually transmitted diseases is human papillomavirus (HPV) infection that affects around 80% of sexually active women. Although most of the women clear HPV infection, individuals with inadequate immune responses develop persistent infections which lead to premalignant lesions such as high-grade cervical intraepithelial neoplasia (CIN-III), with high potential to progress to cervical carcinoma. Based on molecular and epidemiological data, high risk papillomaviruses (hrHPVs) are now recognized as etiologic agents of cervical carcinoma, although some additional events are required [1, 2].

From 200 HPV genotypes identified so far, nearly 40 can infect cervix and at least 15 hrHPVs (16, 18, 45, 31, 52, 33, 58, 35, 59, 51, 56, 39, 68, 73, and 82) are usually associated with the development of carcinomas. Although HPVs 26, 53, and 66 are probably high-risk types limited data link them to cervix cancer [3]. HPV16 accounts for near 60% of the cervical cancer cases in most countries, followed by HPV-18, -31 and -45.

HPVs are small non-enveloped double-stranded DNA viruses of about 8000-base pair long [4]. The viral genome codes for eight proteins and is divided in three functional regions: early (E1-E6), late (L1-L2) and long coding region (LCR). Early genes are synthesized in the infected basal cells and the late ones are expressed in differentiated cells [5], the replication of HPV cycle depending on complete squamous differentiation of the host epithelium. Except for E1 and E2 proteins (necessary for viral replication), papillomaviruses use the host cell machinery for viral DNA synthesis.

New data confirmed that papillomavirus genomes are organized in the form of chromatin-like structures composed by nucleoprotein complexes (nucleosomes) interconnected by a DNA filament. Treatment of CaSki human cervical carcinoma cell line with methidiumpropyl-EDTA-Fe (II) reveals nucleosomes in specific positions on the LCR and

the E6 and E7 genes. First observed by electron microscopy for bovine papillomavirus (BPV) and HPV from human plantar warts, respectively [6], they were subsequently confirmed for CaSki cell line where the localization of nucleosomes was found at LCR, E6 and E7 genes level. The role of nucleosomes is to repress the activity of the E6 promoter. An open chromatin structure (transcriptionally active status) at early and late viral promoter regions was characterized by the presence of dimethylated forms of histone H3K4 as well as acetylated histones H3 and H4 [7]. These observations suggest the involvement of histone modification in HPV transcription regulation.

2. HPV oncogenesis

Most cervical cancers arise at the squamo-columnar junction, a site characterized by continuous metaplastic changes. The highest metaplastic activity occurs in young women who are reported to have the highest incidence of HPV infection. As cervical cancer is frequently detected in women over 35 years, it was suggested that the disease is a consequence of a slow progression of the viral infection acquired at younger age [8].

HPVs complete their life cycle only in fully differentiated squamous epithelium and the presence of viral genomes in the infected cells is essential both for papillomaviruses and their associated pathologies. HPV cervical infections result in three types of clinical manifestations: (a) productive infection which lead to virions production; viral genes expression is strictly associated with host cell differentiation; (b) latent (asymptomatic) infection (characterized by viral genome presence in basal layers) develops within the first three months or it can remain undetected for years [2]; (c) abortive infection, associated in particular with high risk HPV genotypes, arises especially in sites which are non-optimal for productive infection. Squamous and glandular carcinomas do not support the productive program.

Both *in vivo* and *in vitro* studies associated cervical cancer with three viral oncogenes (E5, E6 and E7) coded by hrHPVs [9]. HPV-induced carcinogenesis is a complex process characterized by alterations in tumour-suppressor genes. The aberrant function of these genes and the genomic instability determined by HPV viral genes, cumulated with the action of various cofactors, lead to progressive lesions and finally to cancer. Nevertheless, in the absence of persistent infection, the risk of cervical cancer is low [10].

The switch from productive to abortive infection is determined by a deregulated expression of E6 and E7 viral oncogenes in proliferating cells, thus leading to an extended lifespan [11]. The oncoproteins E6 and E7 interfere with tumor suppressors p53 and pRb respectively, and favour cells to overcome senescence barrier. Moreover, these proteins target a growing number of other cellular proteins/ factors. Epidemiologic and experimental data showed that also the E6 and E7 genes of low-risk types interfere with p53 and pRb and under certain circumstances are able to induce cervical neoplasia.

While in the normal viral life cycle, the genome replicates as episomal molecules, the up-regulation of viral oncogenes expression is associated with the HPV genome integration into

the host cell chromosome [12]. As a result of integration, viral E1 and E2 genes are disrupted and their repressive action on E6 and E7 open reading frames is discontinued. Recent data point out that E2hrHPV proteins exhibit new oncogenic properties which rely on their ability to induce abnormal mitoses, leading to either loss or excess of DNA, together with DNA breaks during anaphase [13]. Although HPV integration plays an important role in the progression to cancer, the mechanisms are still unclear. Viral integration seems to confer selective advantage for oncogenes transcription and stability for transcripts encoding the E6 and E7 proteins which affect the key tumor suppressors p53 and pRB [14]. Cytogenetic mapping of multiple integration sites suggests that HPV integration occurs preferentially in chromosomal fragile sites (CFS) [15] although it is under debate whether these sites present a greater susceptibility or accessibility to integration [16]. Sometimes, hrHPVs integration occurs within or adjacent to cellular oncogenes like *myc*, *APM1*, *TP63*, *hTERT* [17, 18]. Generally, coding regions are rarely targeted by HPV but gene expression and mRNA structure can be altered by insertion of the strong HPV promoter [14, 19].

Up-regulation of viral oncogenes expression together with loss of inhibitory effects of E2, result in cellular immortalization, deregulated proliferation and increased genomic instability [20]. The viral oncogenes are transcribed by a nucleoprotein complex (enhanceosome) that consists of transcriptional factors (JunB/Fra2) and a chromatin remodelling factor (HMG-I(Y) [21]. SMARCA2, a member of the SWI/SNF family of proteins, similar to the *brhma* (*Brm*) protein from *Drosophila*, is involved in transcriptional regulation of certain genes by altering the chromatin structure. When HPV is present as episome, SMARCA2 associates with E2 promoter and enhances E2 transcriptional activation [22]. SMARCA2 mRNA is a specific target for miR-199a-5p and miR-199a-3p in tumor cell lines, being often silenced in tumor cells at the post-transcriptional levels [23]. That might offer an alternative pathway for E2 gene silencing in HPV transforming infection.

The expression of the HPV oncogenes is necessary and sufficient for the initiation of cervical carcinogenesis, but host genome mutations are needed for malignant progression. In cervical cancer, cells accumulate a wide range of numerical and structural chromosomal abnormalities [24] including lagging chromosomal material, anaphase bridges, and multipolar mitoses [25]. As mentioned, *persistent infection* with HPV could lead, under certain conditions, to the insertion of viral genes into the host genome. As a consequence host defense mechanisms, including methylation machinery are activated [26]. Some viruses can find ways to regulate their gene expression (e.g. by modulating DNA methylation) in order to facilitate persistent infection and circumvent immune system [27]. Viral oncoproteins have the ability to modulate the methylation machinery in order to silent tumor suppressor genes. Studies on biological samples revealed an inverse correlation between the hypomethylation status of LCR region, E6 gene expression and the severity of lesion suggesting that hypomethylation accompanies progression to cancer [28, 29]. The studies of Badal et al. [30] revealed a clonal heterogeneity of methylation status in various regions of the viral genome, thus indicating that methylation of the viral oncogenes in cervical lesions is not an event that causes neoplastic progression but the result of transcriptional activity levels.

As mentioned, cellular transformation is a consequence of persistent infection by hrHPVs that leads to clonal progression of the persistently infected epithelium. Viral oncogenes are essential for carcinogenesis and their expression induces the tumorigenic state which ensures cell survival, essential for viral replication and the spread of progeny [31]. Although the viral oncogenes were intensively studied, new data bring more information about their involvement in cervix carcinogenesis.

3. E6 HPV

High-risk E6 proteins are known for their ability to associate and degrade tumor suppressor p53. E6HPVs inactivate p53 in the infected cells, by inducing its degradation through the ubiquitin–proteasome pathway (Mdm2 E3, E6AP) [32]. This interaction prevents p53 from inducing growth arrest and apoptosis and promotes the perpetuation of damaged DNA during the host cell reproduction. Even both high and low risk E6HPV proteins interact with p53, only high risk oncogenes are capable of binding to the core region of p53. This step is mediated by recruitment of E6-AP (E3 ubiquitin ligase) which interacts with the viral oncogene and forms a complex with both E6 and target proteins [33].

E6 may inhibit p53 signalling pathways independent of protein degradation through p53 sequestration in the cytoplasm or by enhancing p53 nuclear export [34]. As a consequence, E6 oncoprotein precludes the growth-suppressive activities of p53 by transcriptional suppression of its target genes. It inhibits p53 activity by abrogation of the p53 transactivation *via* interaction with CBP/p300 [35, 36] or hADA3 histone acetyl-transferases, proteins involved in the regulation of transcription and DNA replication [37][38]. E6hrHPV proteins display a PDZ (*postsynaptic density protein, discs large tumor suppressor, and the epithelial tight junction protein, Z0-1*) motif designated as S/TXV at their C-terminus end which mediates E6 binding to proteins with these specific domains [39]. There are many studies focused on these interactions with proteins involved in proliferation control such as hDlg1 [40], hScrib [41], MAGI, PTPN3, MUPP1 [42, 43]. hScrib functions as a tumor suppressor that negatively regulates proliferation. hDlg and hScrib are proteins associated with cell junctions mediating the adhesion of basal cells to the ECM. Both are targeted for ubiquitination by high risk E6–E6AP complex, thus affecting epithelial cell growth [41]. MAGI proteins (membrane-associated guanylate kinase homologues –MAGUKs) are found at the tight junctions in epithelial cells and are thought to act in signalling pathways. Their degradation by E6 disrupts regulation of epithelial proliferation [42, 43]. Experimental evidence indicates that the interaction of E6 with PDZ proteins is necessary for the development of epithelial hyperplasia [44].

Several proteins involved in apoptosis and immune evasion (Bak, FADD, c-Myc, NFX1, procaspase 8, etc) [45, 46, 47, 48] are also targeted by E6 HPV. Bak, a member of the Bcl-2 family, is a proapoptotic protein whose interaction with the viral oncogene leads to the inhibition of apoptosis. This strategy by which the virus circumvents apoptosis might contribute to its oncogenic potential.

E6 oncoprotein is also able to modulate transcription from other cellular signaling pathways by interacting with three G-protein (E6TP, Gps2, Tuberin). E6 binds and degrades E6TP1 (E6-

targeted protein 1) in an E6AP dependent manner [49]. E6TP1 has homology to GAPs (*GTPase activating proteins*) for Rap [50] and its interaction with E6 was observed only in cancer-associated high-risk HPV but not in lesion-associated low-risk HPV. E6TP1 is involved in regulating cell proliferation and malignant cellular transformation, and its degradation by ubiquitination seems to be related to cellular immortalization suggesting a critical role of functional inactivation of E6TP1 in E6-induced cellular immortalization.

Tuberin is another protein with GAP activity which is degraded by E6. Tuberin functions in the hamartin-tuberin complex, which exhibits GAP activity toward Rheb protein. This complex is a negative regulator of mTOR signaling [51]. E6 also binds and degrades Gps2, that is involved in suppressing G-protein signaling pathway and c-Jun N-terminal kinase (JNK) activity [52].

E6 is involved in the blockage of apoptosis acting in both major apoptotic pathways:

a. the extrinsic pathway, which triggers extracellular signals that induce the activation of “death receptors” on the cell surface: 1) E6 binds to the death receptor TNFR-1, inhibiting TNFR-1 association with the TRADD (*TNFR1-associated death domain adapter molecule*) and blocking TNFR-1 death domain mediated apoptosis [47]; 2) E6 inhibits apoptosis by binding and degrading both the FADD adapter protein and caspase-8 [53].

b. the intrinsic pathway, which triggers sensing apoptotic signals that arise within the cell (DNA damage, oxidative stress) [54]. The Lr/hrE6 oncoproteins block intrinsic apoptotic signaling interacting with Bak, inducing its proteasomal-dependent degradation [55], or using a mechanism depending or not on E6AP and E3 ubiquitin ligases [56].

Another biological activity of E6 oncoprotein consists in alteration of cell adhesion in order to allow proliferation of differentiated cells and inhibition of terminal differentiation: extracellular matrix adhesion, cell:cell contact and cytoskeletal organization. For example, Paxillin and zyxin are focal adhesion molecules involved in binding cellular cytoskeleton to the ECM and transmitting signals along the actin network from the ECM to nucleus. Focal adhesions form a structural link between the extracellular matrix and the actin cytoskeleton, and are important sites of signal transduction. Paxillin has been shown to bind to β -integrin, oncoproteins such as v-Src, v-Crk, p210BCR/ABL, p125FAK, vinculin, and talin, and is involved in changing the organization of the actin cytoskeleton. E6HPV interacts with paxillin [57], and the binding leads to the disruption of the actin cytoskeleton, a characteristic of many transformed cells.

E6 interacts with proteins involved in chromosomal stability within the HPV infected cell. Viral protein mediates MCM7 (*minichromosome maintenance 7*) degradation via E6AP and leads to chromosomal abnormalities in HPV infected cells [58]. Moreover, its interactions with XRCC1 and O(6)-methylguanine-DNA methyltransferase (MGMT) (proteins involved in single strand DNA break repair) induce DNA damage [59] or sensitize HPV infected cells to alkylating DNA damage [60]. These interactions increase genomic instability and accelerate the progression to carcinogenesis.

E6 hrHPV is also involved in immune evasion by interacting with IFR-3 (*Interferon regulatory factor-3*) [61], that is required for the expression of type I interferon. IFR-3 is activated by virus infection to form a complex with transcriptional regulators of the IFN-beta. Therefore IFR-3 inhibition affects its transactivation ability and results in the induction of IFN- β following viral infection [62]. Interferon induced growth arrest is dependent on p53 acetylation, a modification which affects p53 stability and increases its transcriptional activity. As E6 targets p53 directly or through p300/CBP, the virus induces proliferation of HPV infected cells in the presence of interferon [63]. Moreover, E6 inhibits TLR9 transcription, leading to a functional loss of TLR9 signaling pathways within the cell [61]. By activating telomerase, E6 promotes malignancy as the mutant cells continue to reproduce uncontrollably [64]

It was noticed that oncogenic E6HPV is able to modulate the expression of many cellular miRNAs *via* p53. For example, miR-34a gene is a direct transcriptional target of p53 and its expression is transactivated by p53 binding to a consensus p53 binding site in the miR-34a promoter region [65, 66, 67]. As E6 is a regulator of p53, viral oncoprotein leads to the reduction of miR-34a, and this affects the expression of cell cycle regulators, including cyclin E2, cyclin D1, CDK4, CDK6, Bcl-2, SIRT1, and p18Ink4c [68, 69]. The hypothesis that p53 modulates cellular miRNAs down-regulation mediated by E6HPV16 was sustained by the fact that the genes encoding miR-34a and miR-23b contain a promoter region with a p53 binding site [70].

4. E7HPV

The HPV life cycle is associated with the differentiation process of the infected epithelial cell. Interaction between E7 and pRB determines degradation and phosphorylation of pRB with the release of E2F and activation of genes that promote cellular proliferation. The actions of E7 induce cells to enter in the S phase of cell cycle (including suprabasal epithelial cells) which ensures all cellular factors necessary for viral replication. On the other hand, E7 can directly bind E2F1 and enhance E2F1-mediated transcription [71]. E2F transcription factors are critical regulators of G1 exit and S-phase progression. In addition, cellular differentiation, apoptosis and genomic instability are controlled by E2Fs [72]. E7 also interacts with pRb associated proteins (p107, p130) which are negative regulators of the cell cycle involved in G1/S and G2/M transition, via the LXCXE motif in CR2 [73].

E7hrHPV can associate and alter the activities of multiple cellular factors that normally contribute to the regulation of the cell cycle. In addition to targeting pRB for proteasomal degradation [74] E7 inhibits p21 functions by direct binding, thus contributing to sustained activity of CDK [73, 75, 76]. Cyclin dependent kinases (cdks) are the most important in cell division cycle. Expression of cyclins E and A, the regulatory subunits of cdk2, which drives S-phase entry and progression, is under E2F control and they are both expressed at higher levels in E7 expressing cells [77].

A cell infected by virus will usually respond by producing interferons (IFNs) that have an antiviral and antitumour effect. HPV16 E7 protein has been shown to block IFN- α activity

and inhibit IFN- β promoter [78]. Besides its role in cell proliferation E7 also regulates apoptosis. Some studies underlined that the actions of E7 appear to be anti-apoptotic. On the other hand, overexpression of E7 in genital keratinocytes induces spontaneous cell death. However, its effect on cellular apoptotic pathways is pleiomorphic.

E7hrHPV increases genomic instability in primary human cells [79] and generates mitotic defects and aneuploidy as a consequence of gains or losses of entire chromosomes during mitosis, or by induction of supernumerary centrosomes and multipolar mitoses [25]. Supernumerary centrosomes and associated multipolar mitoses have been detected in cells that express low copy numbers of episomal E7HPV [80] and their incidence increases in cells with integrated HPV, presumably due to higher E7 expression. Induced aberrant centriole synthesis is dependent on cdk2 activity in E7 expressing cells [81, 82]. The ability of HPV16 E7 to induce supernumerary centrosomes is at least in part independent from the ability to target pRB family members. A possible pRB/p107/p130 independent mechanism involves the association of E7 with the centrosomal regulator γ -tubulin [83]. E7HPV expression also causes other types of mitotic abnormalities including lagging chromosomal material and anaphase bridges that may represent chromosomal fusions caused by double strand DNA breaks [84]. The presence of DNA repair foci indicates that E7 may induce double strand DNA breaks or interfere with break repair. This may facilitate viral genome integration.

High-risk E7 proteins are also able to direct the interaction with chromatin modifiers such as histone deacetylases HDACs 1, 2, 3 and 8, events independent of pRB inactivation. HDACs are involved in regulation of transcription of different genes, including those that present promoters activated by E2F transcriptional factor [84]. This interaction additionally affects the expression of S phase genes. Experimental studies showed that L67 mutation of HPV31 E7 protein determines the impossibility to bind HDACs, and affects the virus ability to remain in an episomal status or to complete the later stages of the viral life cycle [85]. Currently, there is no study to prove the connection between the viral replication and the binding of E7 to HDACs. E7hrHPV also interacts with c-myc, augments c-myc transactivation, contributing to an efficient immortalization.

As we mentioned before, HPV16 E7 oncoprotein interacts with complexes formed between E2F6 and polycomb transcriptional repressor (PRCs) [86]. PRCs associate with histone H3 lysine 27 trimethyl (H3K27me3) [87] and inactivate p14ARF tumor suppressor [88]. Highly HPV16E7 expression correlates with high levels of H3K27me3 [89] and an increased expression of p16INK4A through KDM6B induction. This process is not dependent of pRB inactivation by E7 oncogene, but several genes (Hox) regulated by KDM6A or KDM6B are highly expressed, promoting cell proliferation and escape from senescence. Gathering these data, HPV16E7 expression may cause epigenetic reprogramming in host cells, inducing alterations in H3K27me3 levels and transcriptional changes. On the other hand, KDM5 has been linked to oncogenesis process [86]: E2 protein recruits KDM5 in order to repress the transcription of the E6 and E7 oncoproteins through the HPV LCR [90, 91]. The interplay between histone methyl-transferases and demethylases in the context of viral oncogenes activity is shown in figure 1.

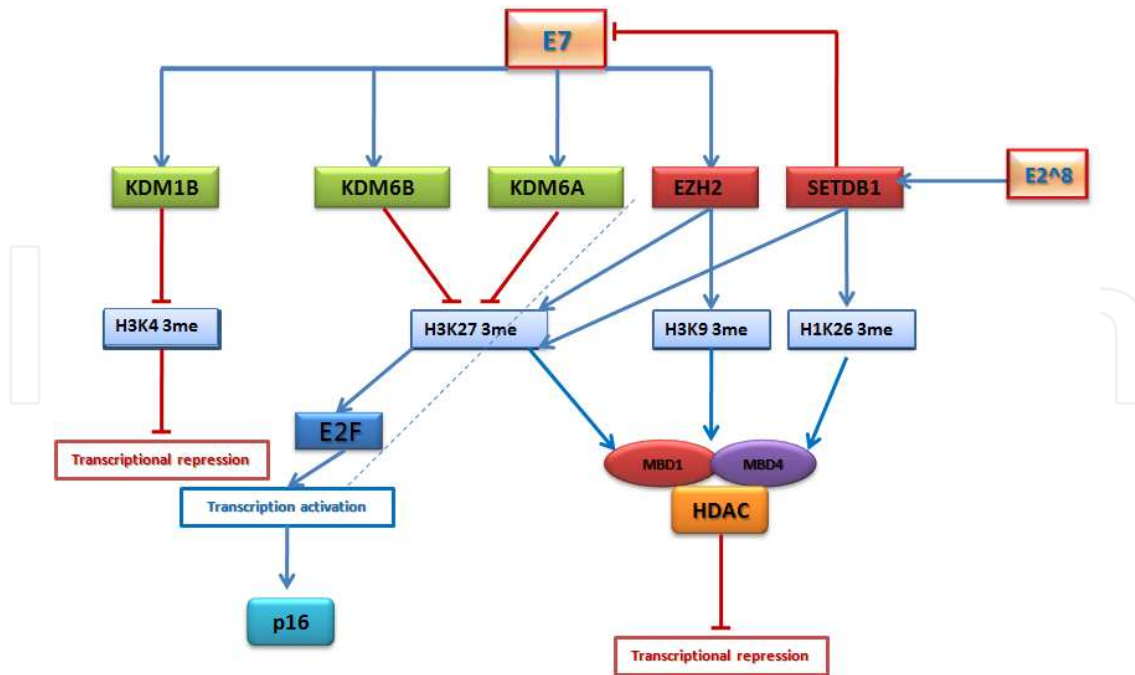


Figure 1. The interplay between histone methyl-transferases and demethylases in the context of viral oncogenes activity

Expression of both early and late HPV genes seems to be subject to miRNA-mediated regulation at the post-transcriptional level in cervical cancer cell lines [68, 69].

E7 increases the expression of miR-15/16 cluster through pRB degradation and release of E2, a factor which promotes miR-15a/16-1 cluster expression [68, 69]. On the other hand, E7hrHPV down-regulates the expression of miR-203 [92], a micromolecule involved in the control of the keratinocytes differentiation by shift from a proliferative to a nonproliferative status [93, 94]. Another cause of miR-203 down-regulation is due to methylation of CpG islands in the promoter region. Hypermethylation of miR203 as well as miR-34b and miR-124 was correlated with CIN III and cervical cancer [95].

Beside the E6 and E7 oncogenes, recent studies emphasize the role of two other viral proteins in HPV induced oncogenesis: E5 and E2.

5. E5 HPV

E5 HPV16 is a hydrophobic protein (83 amino acid long), localized in the intracellular membranes (plasma membrane, endoplasmic reticulum, nuclear envelope and Golgi apparatus) [96]. Based on its interactions with cellular proteins, E5 seems to be a weak transforming protein. These interactions lead to alterations in normal biological activity and evasion of the immune response. E5 gene encodes short hydrophobic peptides, which have mitogenic activity, synergic with EGF (*epidermal growth factor*) [97]. In hrHPV infections, (*e.g.* HPV 16) EGF receptor increases 2-5 times in the human keratinocytes expressing E5, leading to cell proliferation. This suggests that HPV16 E5 plays a major role in expanding populations of HPV16-infected basal keratinocytes *in vivo* by augmenting extracellular

growth signals in viral infected cells (enhancing ligand-dependent EGF-R activation). E5 oncoprotein binds and inhibits the activity of the 16 kDa subunit of vacuolar ATP-ase (V-ATPase), altering the endosomal acidification and degradation of EGF-R [98, 99]. The delay of EGF-R degradation can be reached by E5 interference with membrane trafficking and the fusion of early and late endosomes [100].

E5 activates EGF-R signaling pathway through either EGF-dependent or EGF-independent processes, but it is also capable of interacting with, enhancing/ altering the signaling of other different classes of growth factor receptors like:

- G protein-coupled endothelin receptor [96]; this interaction induces mitogenic activity of ET-1, leading to the chronic stimulation of keratinocyte proliferation;
- keratinocyte growth factor receptor/ fibroblast growth factor receptor 2b (KGF-R/FGF-R2b); these receptors are down-modulated by E5, through reduction of transcripts and protein [101, 102].
- connexin 43 interferes with E5, in order to inhibit gap junction-mediated communication between epithelial cells in monolayer [103] and in raft cultures [104]; this makes the transformed cells more insensitive to homeostatic growth control signals from adjacent normal cells. Moreover, E5 seems to be involved in induction of cell fusion [105, 106, 107] a critical event in the early stage of HPV-associated cervical cancer. There are experimental data which support E5 role in cervical cancer. It seems this viral protein increases the efficiency of keratinocytes immortalization induced by E6 and E7 oncogenes [108] and affects cell-cell communications [109]

In early infection, E5 appears to inhibit programmed cell death [110] using different mechanisms like: (a) down regulation of the total amount of Fas receptor and reduction of Fas surface location; and (b) alteration of the formation of *Death-Inducing Signalling Complex* (DISC) triggered by TRAIL [111]. While E5 did not down-regulate TRAIL receptor expression, it was found to inhibit TRAIL signaling by interfering with the formation of the TRAIL DISC and thereby inhibiting the cleavage of procaspases-3 and -8, as well as of PARP [111]. Therefore, it is possible that E5 interferes with the ability of the immune system to eliminate infected cells by impairing death receptor signaling. Together, the results of these studies provide strong evidence that E5 contributes to the evasion of immune surveillance during the early stages of HPV infection.

In contrast, HPV16E5 sensitizes human keratinocytes to apoptosis induced by osmotic stress, perhaps due to cell membrane modifications caused by this strong hydrophobic molecule [111]. On the other hand, E5 can inhibit the ER stress pathway, cyclooxygenase-2 (COX-2), XBP-1 and IRE1a, but this seems to be limited to the high risk genotypes, favoring viral replication and persistence [112].

The role of E5 in HPV transformation might be due to alteration of innate and adaptive immune responses. E5 protein seems to down-regulate MHC/HLA class I, through alkalinisation of the endomembrane compartments [113] and the direct interaction of E5 with the heavy chain of the MHC class I complex [114, 115]. E5 gene is often deleted during viral DNA integration into the host cell genome. This might suggest a dispensable role of

this gene in oncogenesis but the fact that E5 mRNA is the most abundant viral transcript prior integration sustains its role in early phases of tumorigenesis.

6. E2 HPV

The E2HPV protein exhibits complex functions independent of transcription; it can modulate the host cells in concert with the viral vegetative cycle. Recent data point out that E2HPV could be involved in early carcinogenesis [13]. E2 is a repressor of E6 and E7 transcription in the HPV context. E2HPV is involved in viral transcription and replication [116, 117], forming together with E1 a complex with viral origin of replication and recruiting cellular DNA replication machinery (DNA polymerases, replication protein A, replication protein C, topoisomerase I/II and proliferating-cell nuclear antigen) in order to facilitate viral DNA replication [116]. It was noted that E2 protein is expressed at relatively high levels in differentiated cells of the intermediate layers of CIN lesions; on the other hand, its expression is decreased with progression of the lesions and is absent in most of the cancers *in situ*, being inversely correlated with expression of E7 [13, 118]. E2 is an unstable protein expressed in both the nuclei and cytoplasm of infected cells, and is degraded through the proteasome [119, 120]. In most cases, HPV integration occurs by breaking the E2 gene region. Re-expression of E2 in cervical carcinoma cell lines appears to be detrimental to cell proliferation due to the induction of G1 cell cycle arrest through repression of the endogenous E6/E7 expression, as well as due to induction of cellular senescence and apoptosis [121, 122]. The fact that E2 can regulate the activities of E6 and E7 via transcriptional control or by direct interaction [28], suggests that HPV genome integration may result from a strong selective pressure on the virus to avoid E2-induced apoptosis while modulating the survival of infected cells through the activities of E6 and E7.

E2 could enhance cellular DNA replication through abrogation of a mitotic checkpoint [123] and blocking the cell cycle in G2/M [124]; E2 activates the spindle assembly checkpoint and induces abnormal chromosome segregation after anaphase, leading to aneuploidy or DNA breaks [13]. The potential role of E2 to induce abnormal mitoses links E2 to HPV-associated carcinogenesis. This hypothesis is sustained by the fact that only E2 proteins from high-risk HPVs could induce abnormal mitotic phenotypes, in contrast to the E2 proteins from the low-risk HPV11 and 6 which are inactive [124].

7. Prevention of cervical carcinoma

The high risk human papillomaviruses are associated with cervical cancers and play an essential role in the pathogenesis of the disease. Although commercial prophylactic HPV vaccines are now available, they do not have therapeutic effect against established HPV infections and HPV-associated lesions which account for high morbidity and mortality worldwide. Advanced cervical cancer remains a public health issue despite the availability of preventive vaccines and population-based screening because they target a very young teenage population with a delayed impact on cervical cancer due to the peak of cervical cancer incidence at ages 45–55 [125]. Viral oncoproteins are critical for the induction and maintenance

of cellular transformation in HPV-infected cells. Therefore, E6 and E7 are considered the ideal targets for therapeutic HPV vaccines. By contrast with the commercial preventive HPV vaccines (Gardasil and Cervarix) which use HPV virus-like particles to generate neutralizing antibodies, therapeutic vaccines can eliminate preexisting lesions and infections by generating cellular immunity against HPV-infected cells. Consequently, many therapeutic vaccine strategies have focused primarily on stimulating the production and activation of T cells that can recognize infected cells expressing the target antigens (E6 and E7).

A variety of vaccine strategies have been employed to target immune responses to these proteins. Various therapeutic HPV vaccines for cervical cancer, including live vector-, peptide-, protein-, nucleic acid-, or cell-based vaccines targeting the E6HPV and/or E7 antigens were developed.

Live vector-based vaccines (bacterial and viral vectors) are attractive due to their high immunogenicity and efficiency in delivering antigens or DNA encoding antigens of interest. Among them, ADXS11-001 (*Listeria*-based vaccine) targets E7 and is well tolerated by patients in end-stage of cervical cancer, who had failed prior chemotherapy, radiotherapy and/or surgery (phase I trial in patients with stage IVb cervical cancer) [126]. On the other hand, TA-HPV (a recombinant vaccinia virus expressing HPV-16/18 E6/E7 fusion protein), induces HPV antigen-specific T cell-mediated immune response (phase I/II trial in patients with Ib or IIa cervical cancer) [127]. MVA-E2 and MVA-HPV-IL2 (Modified Vaccinia Ankara-based vaccines expressing HPV16 E6/E7 and IL-2) showed some promises as 50% of the treated patients presented complete healing of lesions and E6 and E7 antigen levels below the detection limit of 6 months post vaccination (phase II trial in patients with CIN III) [128].

Cell-based vaccines (dendritic cells-DCs, modified tumor cells) are highly immunogenic and useful in expressing the relevant tumor antigens. Vaccination with E7-presenting DCs transfected with siRNA targeting Bim (Bcl-2-interacting mediator) was capable of generating a strong E7-specific CTL response and a marked therapeutic effect in vaccinated mice [129]. The vaccine was in clinical pilot study in patients with late stage cervical cancer [130] or phase I trial in patients with stage Ib or IIa cervical cancer [131], or with recurrent cervical cancer [132].

Peptide-based vaccines can combine multiple epitopes and enhance peptides for MHC binding. The peptide-based vaccine potency is increased by using adjuvants such as GM-CSF, 4-1BB ligand, and Montanide ISA 51) [133, 134]. Several vaccines from this category are in different phase trials: Lipopeptide - lipidated E7 (HLA-A* 0201 - restricted epitope, a.a. 86– 93 lipopeptide) in phase I trial in patients with refractory cervical or vaginal cancer; Peptide & Montanide ISA -51 (HLA-A * 0201- HPV16 E7 epitopes restricted, a.a. 12 to 20 ± a.a. 86 -93) ligated to PADRE, adjuvant Montanide ISA 51, in phase I/II trial in patients with recurrent or residual cervical cancer. TriVax- HPV16-E7 epitope (E7 49-57 with CD40 mAb) in tumor-bearing mice [135] has not yet been tested in clinical trials.

The protein-based vaccines take the advantage of using fully purified proteins which are able to induce humoral and cellular immune response. Conformational epitopes of injected

proteins are recognized by B cell receptors, triggering specific immunoglobulin synthesis. Proteins are retrieved and processed by antigen presenting cells (dendritic cells, macrophages, etc.) in cooperation with T helper lymphocytes (by presenting peptides conjugated with MHC class II molecules) and cytotoxic T lymphocytes (by presenting peptides conjugated with MHC class I molecules). Adjuvants (Iscomatrix, AS02B, Poly ICLC) [136, 137] and fusion immunostimulatory proteins (heat shock protein derived from *Mycobacterium bovis*) improve CTL responses of HPV protein-based vaccines [138].

Nucleic acid-based vaccines are based on the direct introduction into the host cell of information encoding the antigen of interest, synthesized *in situ*. Recombinant DNA is introduced into tissues by intramuscular inoculation. Dendritic cells serve as central players for DNA vaccine development. pNGVL4a encodes the signal sequence *Sig* linked to a weakened form of HPV16 E7 fused to HSP70. It is used in phase I trial in patients with CIN II/III [139]. VGX-3100 vaccine expressing E6 and E7 HPV16/18 proteins was used in Phase I trial in patients with CIN II/III post-surgical or ablative treatment [140]. Regarding the potential of E5 to contribute to HPV-associated carcinogenic process, it was suggested its role during the early tumorigenesis stage. Taking into account a limited immune response in malignant stage as a result in down-regulation of MHC class I and II molecules, a therapeutic vaccine targeting E5-expressing cells might be a good strategy to prevent the progression of premalignant lesions toward invasive cervical cancers [141]. Using a recombinant adenovirus which expresses E5HPV16 (AdV-based E5) in syngenic animals, it was observed a reduction of tumor growth correlated with tumor protection through CD8 T cells [142], but HPV16E5 25-33 peptide plus CpG oligodeoxynucleotides (ODN) proved to be more effective as vaccine [143]. The construction of different DNA vectors based on epitopes of E5 HPV oncogene (now under evaluation in animal models) was also described [144]. The immune effector cells in premalignant lesions may eradicate tumor cells more efficiently than in the invasive cervical cancers.

8. Conclusions

The studies of HPV E6 and E7 oncogenes and the proteins coded will continue in order to discover new diagnostic and prognostic tools for cervical cancer. On the other hand, scientific studies will probably decipher new pathways of HPV oncogenesis molecular network and these will enrich the knowledge in this field.

Abbreviations

ABL	Abelson murine leukemia
APM	adipose most abundant gene transcript
Bcl-2	B cell leukemia/lymphoma 2
BCR	breakpoint cluster region
Brm	brahma
CREB	cyclic AMP response element-binding protein
CBP	CREB-binding protein

CDK	cyclin-dependent kinases
CKI and II	casein kinase I and II
COX-2	cyclooxygenase 2
E6-AP	E6-associated protein
E6-BP	E6-binding protein
ET1	etched1
FADD	Fas-associated protein with death domain
FAK	focal adhesion kinase
Fra2	Fos related antigen
GAP	GTPase activating proteins
GM-CSF	granulocyte-macrophage colony-stimulating-factor
Gps2	G-protein pathway suppressor 2
hADA3	transcriptional adaptor 3
HDAC	histone deacetylase
hDlg	human Drosophila discs large protein
hDlg1	discs large homolog 1
hE6TP1	human E6-targeted protein 1
HLA	human leukocyte antigen
HMG	high mobility group
HPV	human papilloma virus
hScrib	human Scribble tumor suppressor protein
hScrib	scribbled homolog Drosophila
hTERT	human telomerase reverse transcriptase
IFN	interferon
IFNAR1	interferon-alpha receptor 1
IRE1a	endoribonuclease/protein kinase IRE1-like protein
IRF-1 and -3	interferon regulatory factor 1 and 3
ISGF3	interferon-stimulated gene factor 3
KDM1	lysine (K)-specific demethylase 1A
M2-PK	M2 pyruvate kinase
MAGI	membrane-associated guanylate kinases
Mdm2	murine double minute 2
MHC	major histocompatibility complex
MMP-7	multicopy maintenance protein 7
mTOR	mechanistic/mammalian target of rapamycin (serine/threonine kinase)
MUPP1	multi-PDZ-domain protein 1
NFX1	nuclear factor (X can be any amino acids)
p18Ink4c	Cyclin-dependent kinase 4 inhibitor C
PTPN3	protein tyrosine phosphatase, non-receptor type 3
Rb	retinoblastoma protein
Rheb	Ras homolog enriched in brain
S4	subunit 4
SIRT	sirtuin (silent mating type information regulation 2 homologue) 1

SMARC SWI/SNF-related, matrix-associated, actin-dependent regulators of chromatin
SWI/SNF Switch/Sucrose nonfermentable
TBP TATA box-binding protein
TLR9 toll-like receptor 9
TNFR1 tumor necrosis factor receptor, member 1
TP63 tumor protein p63
TRADD TNFRSF1A-associated via death domain
TRAIL tumor necrosis factor-related apoptosis-inducing ligand
v- Src sarcoma viral oncogene
v-Crk sarcoma virus CT10 oncogene homolog (avian)-like
XBP-1 X-box binding protein 1
XRCC1 X-ray repair complementing defective repair in Chinese hamster cells 1

Author details

Gabriela Anton, Adriana Plesa, Coralia Bleotu, Anca Botezatu and Mihai Stoian
Viral Genetics Dept, Stefan S Nicolau Institute of Virology, Bucharest-Romania

Mariana Anton
Genetics Dept, Carol Davila University of Medicine and Pharmacy, Bucharest-Romania

Lorelei Irina Brasoveanu
Center of Immunology, Stefan S Nicolau Institute of Virology, Bucharest-Romania

Acknowledgement

PN2 -41030, PN2-41081 and VIASAN 223 Romanian National Grants.

9. References

- [1] Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N. Human Papillomavirus is a Necessary Cause of Invasive Cervical Cancer Worldwide. *Journal of Pathology* 1999;189: 12-19.
- [2] Muñoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ. Epidemiologic Classification of Human Papillomavirus Types Associated with Cervical Cancer. *New England Journal of Medicine* 2003;348: 518-527.
- [3] Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The Causal Relation between Human Papillomavirus and Cervical Cancer. *Journal of Clinical Pathology* 2002;55: 244-65.
- [4] Sinal SH, Woods CR. Human Papillomavirus Infections of the Genital and Respiratory Tracts in Young Children. *Seminars in Pediatric Infectious Diseases* 2005;16: 306-16.
- [5] Molijn A, Kleter B, Quint W, van Doorn LJ. Molecular Diagnosis of Human Papillomavirus (HPV) Infections. *Journal of Clinical Virology*, 2005;32S: S43-S51.

- [6] del Mar Pena L, Laimins LA. Differentiation-Dependent Chromatin Rearrangement Coincides with Activation of Human Papillomavirus Type 31 Late Gene Expression. *Journal of Virology* 2001;75: 10005-10013.
- [7] Wooldridge TR, Laimins LA, Regulation of Human Papillomavirus Type 31 Gene Expression During the Differentiation-Dependent Life Cycle Through Histone Modifications and Transcription Factor Binding. *Virology* 2008;374: 371-380
- [8] Burd EM. Human Papillomavirus and Cervical Cancer. *Clinical Microbiology Reviews* 2003;16: 1-17.
- [9] Narisawa-Saito M, Kiyono T. Basic Mechanisms of High-Risk Human Papillomavirus Induced Carcinogenesis. *Cancer Science*. 2007;98: 1505-1511.
- [10] Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE. Human Papillomavirus Testing in the Prevention of Cervical Cancer. *Journal of the National Cancer Institute*. 2011;103(5): 368-383.
- [11] Clifford GM, Smith JS, Plummer M, Muñoz N, Franceschi S. Human Papillomavirus Types in Invasive Cervical Cancer Worldwide: a Meta-Analysis. *British Journal of Cancer* 2003;88: 63-73.
- [12] Alazawi W, Pett M, Arch B, Scott L, Freeman T, Stanley MA, Coleman N. Changes in Cervical Keratinocyte Gene Expression Associated with Integration of Human Papillomavirus 16. *Cancer Research* 2002;62: 6959-6965.
- [13] Bellanger S, Tan CL, Xue YZ, Teissier S, Thierry F. Tumor Suppressor or Oncogene? A Critical Role of the Human Papillomavirus (HPV) E2 Protein in Cervical Cancer Progression. *American Journal of Cancer Research* 2011;1(3): 373-389.
- [14] Ziegert C, Wentzensen N, Vinokurova S, Kisseljov F, Einkenkel J, Hoeckel M, von Knebel Doeberitz M. A Comprehensive Analysis of HPV Integration Loci in Anogenital Lesions Combining Transcript and Genome-Based Amplification Techniques. *Oncogene* 2003;22: 3977-3984.
- [15] Yu T, Ferber MJ, Cheung TH, Chung TK, Wong YF, Smith DI. The Role of Viral Integration in the Development of Cervical Cancer. *Cancer Genetics and Cytogenetics* 2005;158(1): 27-34.
- [16] Woodman CBJ, Collins SI, Young LS. The Natural History of Cervical HPV Infection: Unresolved Issues. *Nature Reviews Cancer* 2007;7: 11-22.
- [17] Ferber MJ, Thorland EC, Brink AA, Rapp AK, Phillips LA, McGovern R, Gostout BS, Cheung TH, Chung TK, Fu WY, Smith DI. Preferential Integration of Human Papillomavirus Type 18 near the c-myc Locus in Cervical Carcinoma. *Oncogene* 2003;22: 7233-7242.
- [18] Ferber MJ, Montoya DP, Yu C, Aderca I, McGee A, Thorland EC, Nagorney DM, Gostout BS, Burgart LJ, Boix L, Bruix J, McMahon BJ, Cheung TH, Chung TK, Wong YF, Smith DI, Roberts LR. Integrations of the Hepatitis B Virus (HBV) and Human Papillomavirus (HPV) into the Human Telomerase Reverse Transcriptase (hTERT) Gene in Liver and Cervical Cancers. *Oncogene* 2003;22: 3813-3820.
- [19] Wentzensen N, Vinokurova S, von Knebel Doeberitz. Systematic Review of Genomic Integration Sites of Human Papillomavirus Genomes in Epithelial Dysplasia and Invasive Cancer of the Female Lower Genital Tract. *Cancer Research* 2004;64: 3878-3884.

- [20] Pett M, Coleman N. Integration of High-Risk Human Papillomavirus: a Key Event in Cervical Carcinogenesis? *Journal of Pathology* 2007;212(4): 356-367.
- [21] Bouallaga I, Teissier S, Yaniv M, Thierry F. HMG-I(Y) and the CBP/p300 Coactivator are Essential for Human Papillomavirus Type 18 Enhanceosome Transcriptional Activity. *Molecular and Cellular Biology* 2003;23: 2329-2340.
- [22] Kumar RA, Naidu SR, Wang X, Imbalzano AN, Androphy EJ. Interaction of Papillomavirus E2 Protein with the Brm Chromatin Remodeling Complex Leads to Enhanced Transcriptional Activation. *Journal of Virology* 2007;81: 2213-2220.
- [23] Sakurai K, Furukawa C, Haraguchi T, Inada K, Shiogama K, Tagawa T, Fujita S, Ueno Y, Ogata A, Ito M, Tsutsumi Y, Iba H. MicroRNAs miR-199a-5p and -3p Target the Brm Subunit of SWI/SNF to Generate a Double-Negative Feedback Loop in a Variety of Human Cancers. *Cancer Research* 2011;71(5): 1680-9.
- [24] Mitelman F, Johansson B, Mertens F (2007) Mitelman Database of Chromosome Aberrations in Cancer. <http://cgap.nci.nih.gov/Chromosomes/Mitelman>.
- [25] Duensing S, Munger K. The Human Papillomavirus type 16 E6 and E7 Oncoproteins Independently Induce Numerical and Structural Chromosome Instability. *Cancer Research* 2002;62(23): 7075-7082
- [26] Tao Q, Huang H, Geiman TM, Lim CY, Fu L, Qiu GH, Robertson KD. (2002) Defective *de novo* Methylation of Viral and Cellular DNA Sequences in ICF Syndrome Cells. *Human Molecular Genetics* 2002;11: 2091-2102.
- [27] Tsai CN, Tsai CL, Tse KP, Chang HY, Chang YS. The Epstein-Barr Virus Oncogene Product, Latent Membrane Protein 1, Induces the Downregulation of E-Cadherin Gene Expression via Activation of DNA Methyltransferases. *Proceedings of the National Academy of Sciences of the USA*. 2002;99: 10084-10089.
- [28] Badal V, Chuang LS, Tan EH, Badal S, Villa LL, Wheeler CM, Li BF, Bernard HU. CpG Methylation of Human Papillomavirus Type 16 DNA in Cervical Cancer Cell Lines and in Clinical Specimens: Genomic Hypomethylation Correlates with Carcinogenic Progression. *Virology* 2003;77: 6227-6234.
- [29] Kalantari M, Calleja-Macías IE, Tewari D, Hagmar B, Lie K, Barrera-Saldaña HA, Wiley DJ, Bernard HU. Conserved Methylation Patterns of Human Papillomavirus Type 16 DNA in Asymptomatic Infection and Cervical Neoplasia. *Journal of Virology* 2004;78: 12762-12772.
- [30] Badal S, Badal V, Calleja-Macías IE, Kalantari M, Chuang LS, Li BF, Bernard HU. The Human Papillomavirus-18 Genome is Efficiently Targeted by Cellular DNA Methylation. *Virology* 2004;324: 483-492.
- [31] Garnett TO, Duerksen-Hughes PJ. Modulation of Apoptosis by Human Papillomavirus (HPV) Oncoproteins. *Archives of Virology* 2006;151: 2321-2335.
- [32] Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 Oncoprotein Encoded by Human Papillomavirus Types 16 and 18 Promotes the Degradation of p53. *Cell* 1990;63(6): 1129-1136.
- [33] Schwarz SE, Rosa JL, Scheffner M. Characterization of Human hect Domain Family Members and Their Interaction with UbcH5 and UbcH7. *Journal of Biological Chemistry* 1998;273(20): 12148-12154.

- [34] Mantovani F, Banks L. The Human Papillomavirus E6 Protein and its Contribution to Malignant Progression. *Oncogene* 2001;20(54): 7874-7887.
- [35] Patel D, Huang SM, Baglia LA, McCance DJ. The E6 Protein of Human Papillomavirus Type 16 Binds to and Inhibits Co-activation by CBP and p300. *EMBO Journal* 1999;18(18): 5061-5072.
- [36] Zimmermann H, Koh CH, Degenkolbe R, O'Connor MJ, Muller A, Steger G, Chen JJ, Lui Y, Androphy E, Bernard HU. Interaction with CBP/p300 Enables the Bovine Papillomavirus Type 1 E6 Oncoprotein to Downregulate CBP/p300-Mediated Transactivation by p53. *Journal of General Virology* 2000;81(11): 11-23.
- [37] Kumar A, Zhao Y, Meng G, Zeng M, Srinivasan S, Delmolino LM, Gao Q, Dimri G, Weber GF, Wazer DE, Band H, Band V. Human Papillomavirus Oncoprotein E6 Inactivates the Transcriptional Coactivator Human ADA3. *Molecular and Cellular Biology* 2002;22 (16): 5801-5812.
- [38] Chen JJ, Hong Y, Rustamzadeh E, Baleja JD, Androphy EJ. Identification of an Alpha Helical Motif Sufficient for Association with Papillomavirus E6. *Journal of Biological Chemistry* 1998;273(22): 13537-13544.
- [39] Tong X, Howley PM. The Bovine Papillomavirus E6 Oncoprotein Interacts with Paxillin and Disrupts the Actin Cytoskeleton. *Proceedings of the National Academy of Sciences of the USA* 1997;94(9): 4412-4417.
- [40] Kiyono T, Hiraiwa A, Fujita M, Hayashi Y, Akiyama T, Ishibashi M. Binding of High Risk Human Papillomavirus E6 Oncoproteins to the Human Homologue of the Drosophila Discs Large Tumor Suppressor Protein. *Proceedings of the National Academy of Sciences of the USA* 1997;94(21): 11612-11616.
- [41] Nakagawa S, Huibregtse JM. Human Scribble (Vartul) is Targeted for Ubiquitin-Mediated Degradation by the High-Risk Papillomavirus E6 Proteins and the E6AP Ubiquitin-Protein Ligase. *Molecular and Cellular Biology* 2000;20(21): 8244-8253.
- [42] Jing M, Bohl J, Brimer N, Kinter M, Vande Pol SB. Degradation of Tyrosine Phosphatase PTPN3 (PTPH1) by Association with Oncogenic Human Papillomavirus E6 Proteins. *Journal of Virology* 2007;1(5): 2231-2239.
- [43] Spanos WC, Geiger J, Anderson ME, Harris GF, Bossler AD, Smith RB, Klingelutz AJ, Lee JH. Deletion of the PDZ Motif of HPV16 E6 Preventing Immortalization and Anchorage-Independent Growth in Human Tonsil Epithelial Cells. *Head Neck* 2008;30 (2): 139-147.
- [44] Nguyen ML, Nguyen MM, Lee D, Griep AE, Lambert PF. The PDZ Ligand Domain of the Human Papillomavirus Type 16 E6 Protein is Required for E6's Induction of Epithelial Hyperplasia in vivo. *Journal of Virology* 2003;77(12): 6957-64.
- [45] Spanos WC, Hoover A, Harris GF, Wu S, Strand GL, Anderson ME, Klingelutz AJ, Hendriks W, Bossler AD, Lee JH. The PDZ Binding Motif of Human Papillomavirus Type 16 E6 Induces PTPN13 Loss, which Allows Anchorage Independent Growth and Synergizes with ras for Invasive Growth. *Journal of Virology* 2008;82(5): 2493-2500.
- [46] Degenhardt YY, Silverstein S. Interaction of Zyxin, a Focal Adhesion Protein, with the e6 Protein from Human Papillomavirus Type 6 Results in its Nuclear Translocation. *Journal of Virology* 2001;75(23): 11791-11802.

- [47] Filippova M, Song H, Connolly JL, Dermody TS, Duerksen-Hughes PJ. The Human Papillomavirus 16 E6 Protein Binds to Tumor Necrosis Factor (TNF) R1 and Protects Cells from TNF-Induced Apoptosis. *Journal of Biological Chemistry* 2002;277(24): 21730-21739.
- [48] Filippova M, Johnson MM, Bautista M, Filippov V, Fodor N, Tungteakkhun SS, Williams K, Duerksen-Hughes PJ. The Large and Small Isoforms of Human Papillomavirus Type 16 E6 Bind to and Differentially Affect Procaspase 8 Stability and Activity. *Journal of Virology* 2007;81(8): 4116-4129.
- [49] Lee C, Wooldridge TR, Laimins LA. Analysis of the Roles of E6 Binding to E6TP1 and Nuclear Localization in the Human Papillomavirus Type 31 Life Cycle. *Virology* 2007;358(1): 201-210.
- [50] Gao Q, Srinivasan S, Boyer SN, Wazer DE, Band V. The E6 Oncoproteins of High-Risk Papillomaviruses Bind to a Novel Putative GAP Protein, E6TP1, and Target it for Degradation. *Molecular and Cellular Biology* 1999;19(1): 733-744.
- [51] Huang J, Manning BD. The TSC1-TSC2 Complex: a Molecular Switchboard Controlling Cell Growth. *Biochemical Journal* 2008;412 (2): 179-190.
- [52] Degenhardt YY, Silverstein SJ. Gps2, a Protein Partner for Human Papillomavirus E6 Proteins. *Journal of Virology* 2001;75(1): 151-160.
- [53] Garnett TO, Filippova M, Duerksen-Hughes PJ. Accelerated Degradation of FADD and Procaspase 8 in Cells Expressing Human Papilloma Virus 16 E6 Impairs TRAIL-Mediated Apoptosis. *Cell Death and Differentiation* 2006;13(11): 1915-1926.
- [54] Wang X. The Expanding Role of Mitochondria in Apoptosis. *Genes and Development* 2001;15(22): 2922-2933.
- [55] Underbrink MP, Howie HL, Bedard KM, Koop JI, Galloway DA. The E6 Proteins from Multiple Beta HPV Types Degrade Bak and Protect Keratinocytes from Apoptosis after UVB Irradiation. *Journal of Virology* 2008;82(21): 10408-10417.
- [56] Simmonds M, Storey A. Identification of the Regions of the HPV 5 E6 Protein Involved in Bak Degradation and Inhibition of Apoptosis. *International Journal of Cancer* 2008;123(10): 2260-2266.
- [57] Tong X, Howley PM. The Bovine Papillomavirus E6 Oncoprotein Interacts with Paxillin and Disrupts the Actin Cytoskeleton. *Proceedings of the National Academy of Sciences of the USA* 1997;94(9): 4412-4417.
- [58] Kuhne C, Banks L. E3-Ubiquitin Ligase/E6-AP Links Multicopy Maintenance Protein 7 to the Ubiquitination Pathway by a Novel Motif, the L2G Box. *Journal of Biological Chemistry* 1998;273(51): 34302-34309.
- [59] Iftner T, Elbel M, Schopp B, Hiller T, Loizou JI, Caldecott KW, Stubenrauch F. Interference of Papillomavirus E6 Protein with Single-Strand Break Repair by Interaction with XRCC1. *EMBO Journal* 2002;21(17): 4741-4748.
- [60] Srivenugopal KS, Ali-Osman F. The DNA Repair Protein, O(6)-Methylguanine-DNA Methyltransferase is a Proteolytic Target for the E6 Human Papillomavirus Oncoprotein. *Oncogene* 2002;21(38): 5940-5945.
- [61] Hasan UA, Bates E, Takeshita F, Biliato A, Accardi R, Bouvard V, Mansour M, Vincent I, Gissmann L, Iftner T, Sideri M, Stubenrauch F, Tommasino M. TLR9 Expression and

- Function is Abolished by the Cervical Cancer-Associated Human Papillomavirus Type 16. *Journal of Immunology* 2007;178 (5): 3186-3197.
- [62] Ronco LV, Karpova AY, Vidal M, Howley PM. Human Papillomavirus 16 E6 Oncoprotein Binds to Interferon Regulatory Factor-3 and Inhibits its Transcriptional Activity. *Genes and Development* 1998;12: 2061-2072.
- [63] Beglin M, Melar-New M, Laimins L. Human Papillomaviruses and the Interferon Response. *Journal of Interferon and Cytokine Research*. 2009;29(9): 629-35.
- [64] Riley RR, Duensing S, Brake T, Münger K, Lambert PF, Arbeit JM. Dissection of Human Papillomavirus E6 and E7 Function in Transgenic Mouse Models of Cervical Carcinogenesis. *Cancer Research* 2003;63: 4862-71.
- [65] He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, Xue W, Zender L, Magnus J, Ridzon D, Jackson AL, Linsley PS, Chen C, Lowe SW, Cleary MA, G.J. Hannon GJ. A MicroRNA Component of the p53 Tumour Suppressor Network. *Nature* 2007;447: 1130-1134.
- [66] Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, Feldmann G, Yamakuchi M, Ferlito M, Lowenstein CJ, Arking DE, Beer MA, Maitra A, Mendell JT. Transactivation of miR-34a by p53 Broadly Influences Gene Expression and Promotes Apoptosis. *Molecular Cell* 2007;26: 745-752.
- [67] Raver-Shapira N, Marciano E, Meiri E, Spector Y, Rosenfeld N, Moskovits N, Bentwich Z, Oren M. Transcriptional Activation of miR-34a Contributes to p53-Mediated Apoptosis. *Molecular Cell*. 2007;26: 731-743.
- [68] Zheng ZM. Regulation of Cellular miRNA Expression by Human Papillomaviruses. *Biochimica et Biophysica Acta*. 2011;1809: 668-677
- [69] Wang X, Tang S, Le SY, Lu R, Rader JS, Meyers C, Zheng ZM. Aberrant Expression of Oncogenic and Tumor-Suppressive MicroRNAs in Cervical Cancer is Required for Cancer Cell Growth. *PLoS One*. 2008;3(7): e2557
- [70] Au Yeung CL, Tsang TY, Yau PL, Kwok TT. Human Papillomavirus Type 16 E6 Induces Cervical Cancer Cell Migration Through the p53/MicroRNA-23b/Urokinase-Type Plasminogen Activator Pathway. *Oncogene* 2011;30: 2401-2410.
- [71] Hwang SG, Lee D, Kim J, Seo T, Choe J. Human Papillomavirus Type 16 E7 Binds to E2F1 and Activates E2F1-Driven Transcription in a Retinoblastoma Protein Independent Manner. *Journal of Biological Chemistry* 2002;277(4): 2923-2930.
- [72] Dyson N. The Regulation of E2F by pRB-Family Proteins. *Genes and Development* 1998;12(15): 2245-2262.
- [73] Dyson N, Guida P, Munger K, Harlow E. Homologous Sequences in Adenovirus E1A and Human Papillomavirus E7 Proteins Mediate Interaction with the Same Set of Cellular Proteins. *Journal of Virology* 1992;66(12): 6893-6902.
- [74] Jones DL, Münger K. Analysis of the p53-Mediated G1 Growth Arrest Pathway in Cells Expressing the Human Papillomavirus Type 16 E7 Oncoprotein. *Journal of Virology* 1997;71: 2905-12.
- [75] Tommasino M, Adamczewski JP, Carlotti F., Barth CF, Manetti R, Contorni M, Cavalieri F, Hunt T, Crawford L. HPV16 E7 Protein Associates with the Protein Kinase p33CDK2 and Cyclin A. *Oncogene* 1993;8(1): 195-202.

- [76] McIntyre M.C, Frattini MG, Grossman SR, Laimins LA. Human Papillomavirus Type 18 E7 Protein Requires Intact Cys-X-X-Cys Motifs for Zinc Binding, Dimerization, and Transformation but not for Rb Binding. *Journal of Virology* 1993;67: 3142-3150.
- [77] Zerfass K, Schulze A, Spitkovsky D, Friedman V, Henglein B, Jansen-Durr P. Sequential Activation of Cyclin E and Cyclin A Gene Expression by Human Papillomavirus Type 16 E7 Through Sequences Necessary for Transformation. *Journal of Virology* 1995;69(10): 6389-6399.
- [78] Park JS, Kim EJ, Kwon HJ, Hwang ES, Namkoong SE, Um SJ. Inactivation of Interferon Regulatory Factor-1 Tumor Suppressor Protein by HPV E7 Oncoprotein. Implication for the E7-Mediated Immune Evasion Mechanism in Cervical Carcinogenesis. *Journal of Biological Chemistry*. 2000;275: 6764-6769.
- [79] White AE, Livanos EM, Tlsty TD. Differential Disruption of Genomic Integrity and Cell Cycle Regulation in Normal Human Fibroblasts by the HPV Oncoproteins. *Genes and Development* 1994;8(6): 666–677.
- [80] Duensing S, Duensing A, Flores ER, Do A, Lambert PF, Munger K. Centrosome Abnormalities and Genomic Instability by Episomal Expression of Human Papillomavirus Type 16 in Raft Cultures of Human Keratinocytes. *Journal of Virology* 2001;75(16): 7712-7716.
- [81] Duensing S, Duensing A, Lee DC, Edwards KM, Pibooniyom SO, Manuel E, Skaltsounis L, Meijer L, Munger K. Cyclin-Dependent Kinase Inhibitor Indirubin-3'-Oxime Selectively Inhibits Human Papillomavirus Type 16 E7-Induced Numerical Centrosome Anomalies. *Oncogene* 2004;23(50): 8206-8215.
- [82] Duensing A, Liu Y, Tseng M, Malumbres M, Barbacid M, Duensing S. Cyclin Dependent K2 is Dispensable for Normal Centrosome Duplication but Required for Oncogene-Induced Centrosome Overduplication. *Oncogene* 2006;25(20): 2943-2949.
- [83] Nguyen CL, Eichwald C, Nibert ML, Munger K. Human Papillomavirus Type 16 E7 Oncoprotein Associates with the Centrosomal Component Gamma-tubulin. *Journal of Virology* 2007;81(24): 13533-13543.
- [84] de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone Deacetylases (HDACs): Characterization of the Classical HDAC Family. *Biochemical Journal* 2003;370: 737-749.
- [85] Longworth MS, Laimins LA. The Binding of Histone Deacetylases and the Integrity of Zinc Finger-Like Motifs of the E7 Protein are Essential for the Life Cycle of Human Papillomavirus Type 31. *Journal of Virology* 2004;78: 3533-3541.
- [86] McLaughlin-Drubin ME, Huh KW, Münger K. Human Papillomavirus Type 16 E7 Oncoprotein Associates with E2F6. *Journal of Virology* 2008;82: 8695-8705
- [87] Schwartz YB, Pirrotta V. Polycomb Silencing Mechanisms and the Management of Genomic Programmes. *Nature Reviews Genetics* 2007;8: 9-22.
- [88] Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras Provokes Premature Cell Senescence Associated with Accumulation of p53 and p16INK4a. *Cell* 1997;88: 593-602.
- [89] Agger K. The H3K27me3 Demethylase JMJD3 Contributes to the Activation of the INK4A-ARF Locus in Response to Oncogene- and Stress-Induced Senescence. *Genes and Development* 2009;23: 1171-1176.

- [90] Hayami S, Yoshimatsu M, Veerakumarasivam A, Unoki M, Iwai Y, Tsunoda T, Field HI, Kelly JD, Neal DE, Yamaue H, Ponder BA, Nakamura Y, Hamamoto R. Overexpression of the JmJc Histone Demethylase KDM5B in Human Carcinogenesis: Involvement in the Proliferation of Cancer Cells Through the E2F/RB Pathway. *Molecular Cancer*. 2010;9: 59-72.
- [91] Smith JA, White EA, Sowa ME Powell ML, Ottinger M, Harper JW, Howley PM. Genome-Wide siRNA Screen Identifies SMCX, EP400, and Brd4 as E2-Dependent Regulators of Human Papillomavirus Oncogene Expression. *Proceedings of the National Academy of Sciences of the USA* 2010;107, 3752-3757.
- [92] Melar-New M, Laimins LA. Human Papillomaviruses Modulate Expression of MicroRNA 203 upon Epithelial Differentiation to Control Levels of p63 Proteins. *Journal of Virology* 2010;84: 5212-5221.
- [93] Yi R, Poy MN, Stoffel M, Fuchs E. A Skin MicroRNA Promotes Differentiation by Repressing 'Stemness'. *Nature* 2008;452: 225-229
- [94] Lena AM, Shalom-Feuerstein R, Rivetti D, Aberdam VD, Knight RA, Melino G, Candi E. miR-203 Represses 'Stemness' by Repressing DeltaNp63. *Cell Death and Differentiation* 2008;15: 1187-1195.
- [95] Botezatu A, Goia-Rusanu CD, Iancu IV, Huica I, Plesa A, Socolov D, Ungureanu C, Anton G. Quantitative Analysis of the Relationship between MicroRNA-124a, -34b and -203 Gene Methylation and Cervical Oncogenesis. *Molecular Medicine Reports* 2011;4(1): 121-128.
- [96] Venuti A, Salani D, Poggiali F, Manni V, Bagnato A. The E5 Oncoprotein of Human Papillomavirus Type 16 Enhances Endothelin-1-Induced Keratinocyte Growth. *Virology* 1998;248: 1-5.
- [97] Bouvard V, Matlashewski G, Gu ZM, Storey A, Banks L. The Human Papillomavirus Type 16 Gene Cooperates with the E7 Gene to Stimulate Proliferation of Primary Cells and Increases Viral Gene Expression. *Virology* 1994;203: 73-80.
- [98] Straight SW, Hinkle PM, Jewers RJ, McCance DJ. The E5 Oncoprotein of Human Papillomavirus Type 16 Transforms Fibroblasts and Effects the Down-regulation of the Epidermal Growth Factor Receptor in Keratinocytes. *Journal of Virology* 1993;67: 4521-4532.
- [99] DiMaio D, Mattoon D. Mechanisms of Cell Transformation by Papillomavirus E5 Proteins. *Oncogene* 2001;20: 7866-7873
- [100] Thomsen P, van Deurs B, Norrild B, Kayser L. The HPV16 E5 Oncogene Inhibits Endocytic Trafficking. *Oncogene* 2000;19: 6023-6032.
- [101] Belleudi F, Leone L, Purpura V, Cannella F, Scrofani C, Torrisi MR. HPV16 E5 Affects the KGFR/FGFR2b-Mediated Epithelial Growth Through Alteration of the Receptor Expression, Signalling and Endocytic Traffic. *Oncogene* 2011;30(50): 4963-4976.
- [102] Krawczyk E, Supryniewicz FA, Liu X, Dai Y, Hartmann DP, Hanover J, Schlegel R. Koilocytosis: a Cooperative Interaction between the Human Papillomavirus E5 and E6 Oncoproteins. *American Journal of Pathology* 2008;173: 682-688.
- [103] Oelze I, Kartenbeck J, Crusius K, Alonso A. Human Papillomavirus Type 16 E5 Protein Affects Cell-Cell Communication in an Epithelial Cell Line. *Journal of Virology* 1995;69: 4489-4494.

- [104] Tomakidi P, Cheng H, Kohl A, Komposch G, Alonso A. Connexin 43 Expression is Downregulated in Raft Cultures of Human Keratinocytes Expressing the Human Papillomavirus Type 16 E5 Protein. *Cell and Tissue Research*. 2000;301: 323-327.
- [105] Hu L, Ceresa B. Characterization of the Plasma Membrane Localization and Orientation of HPV16 E5 for Cell-Cell Fusion. *Virology* 2009;393: 135-143.
- [106] Hu L, Plafker K, Vorozhko V, Zuna RE, Hanigan MH, Gorbsky GJ, Plafker SM, Angeletti PC, Ceresa BP. Human Papillomavirus E5 Induces Bi-Nucleated Cell Formation by Cell-Cell fusion. *Virology* 2009;384: 125-134.
- [107] Hu L, Potapova TA, Li S, Rankin S, Gorbsky GJ, Angeletti PC, Ceresa BP. Expression of HPV16 E5 Produces Enlarged Nuclei and Polyploidy through Endoreplication. *Virology* 2010;405: 342-351.
- [108] Stoppler MC, Straight SW, Tsao G, Schlegel R, McCance DJ. The E5 Gene of HPV-16 Enhances Keratinocyte Immortalization by Full-Length DNA. *Virology* 1996;223: 251-254.
- [109] Oelze I, Kartenbeck J, Crusius K, Alonso A. Human Papillomavirus Type 16 E5 Protein Affects Cell-Cell Communication in an Epithelial Cell Line. *Journal of Virology* 1995;69: 4489-4494.
- [110] Zhang B, Spandau DF, Roman A. E5 Protein of Human Papillomavirus Type 16 Protects Human Foreskin Keratinocytes from UV B-Irradiation-Induced Apoptosis. *Journal of Virology* 2002;76: 220-231.
- [111] Kabsch K, Alonso A. The Human Papillomavirus Type 16 (HPV-16) E5 Protein Sensitizes Human Keratinocytes to Apoptosis Induced by Osmotic Stress. *Oncogene* 2002;21: 947-953.
- [112] Sudarshan SR, Schlegel R, Liu X. The HPV-16 E5 Protein Represses Expression of Stress Pathway Genes XBP-1 and COX-2 in Genital Keratinocytes. *Biochemical and Biophysical Research Communications*. 2010;399: 617-622.
- [113] Schapiro F, Sparkowski J, Adduci A, Schlegel R, Grinstein S. Golgi Alkalinization by the Papillomavirus E5 Oncoprotein. *Journal of Cellular Biology* 2000;148: 305-315.
- [114] Cortese MS, Ashrafi GH, Campo MS. All Four Di-Leucine Motifs in the First Hydrophobic Domain of the E5 Oncoprotein of Human Papillomavirus Type 16 are Essential for Surface MHC Class I Downregulation Activity and E5 Endomembrane Localization. *International Journal of Cancer* 2010;126(7): 1675-1682.
- [115] Ashrafi GH, Haghshenas M, Marchetti B, Campo MS. E5 Protein of Human Papillomavirus 16 Downregulates HLA Class I and Interacts with the Heavy Chain via its First Hydrophobic Domain. *International Journal of Cancer* 2006;119: 2105-2112.
- [116] Melendy T, Sedman J, Stenlund A. Cellular Factors Required for Papillomavirus DNA Replication. *Journal of Virology* 1995;69: 7857-7867.
- [117] Thierry F. Transcriptional Regulation of the Papillomavirus Oncogenes by Cellular and Viral Transcription Factors in Cervical Carcinoma. *Virology* 2009;384: 375-379.
- [118] Xue Y, Bellanger S, Zhang W, Lim D, Low J, Lunny D, Thierry F. HPV16 E2 is an Immediate Early Marker of Viral Infection, Preceding E7 Expression in Precursor Structures of Cervical Carcinoma. *Cancer Research* 2010;70: 5316-5325.

- [119] Blachon S, Bellanger S, Demeret C, Thierry F. Nucleo-Cytoplasmic Shuttling of High Risk Human Papillomavirus E2 Proteins Induces Apoptosis. *Journal of Biological Chemistry* 2005;280: 36088-36098.
- [120] Bellanger S, Demeret C, Goyat S, Thierry F. Stability of the Human Papillomavirus Type18 E2 Protein is Regulated by a Proteasome Degradation Pathway through its Amino-Terminal Transactivation Domain. *Journal of Virology* 2001;75: 7244-7251.
- [121] Goodwin EC, DiMaio D. Repression of Human Papillomavirus Oncogenes in HeLa Cervical Carcinoma Cells Causes the Orderly Reactivation of Dormant Tumor Suppressor Pathways. *Proceedings of the National Academy of Sciences of the USA* 2000;97: 12513-12518.
- [122] Dowhanick JJ, McBride AA, Howley PM. Suppression of Cellular Proliferation by the Papillomavirus E2 Protein. *Journal of Virology* 1995;69: 7791-7799.
- [123] Frattini MG, Hurst SD, Lim HB, Swaminathan S, Laimins LA. Abrogation of a Mitotic Checkpoint by E2 Proteins from Oncogenic Human Papillomaviruses Correlates with Increased Turnover of the p53 Tumor Suppressor Protein. *EMBO Journal* 1997;16: 318-331.
- [124] Bellanger S, Blachon S, Mechali F, Bonne-Andrea C, Thierry F. High-Risk but not Low-Risk HPV E2 Proteins Bind to the APC Activators Cdh1 and Cdc20 and Cause Genomic Instability. *Cell Cycle* 2005;4: 1608-1615.
- [125] Scholl SM, Kenter G, Kurzeder C, Beuzeboc P. Pathway Profiling and Rational Trial Design for Studies in Advanced Stage Cervical Carcinoma: a Review and a Perspective. *ISRN Oncol.* 2011:403098.
- [126] Maciag PC, Radulovic S, Rothman J. The First Clinical Use of a Live-Attenuated *Listeria Monocytogenes* Vaccine: a Phase I Safety Study of Lm-LLO-E7 in Patients with Advanced Carcinoma of the Cervix. *Vaccine* 2009;27(30): 3975-3983.
- [127] Kaufmann AM, Stern PL, Rankin EM, Sommer H, Nuessler V, Schneider A. Safety and Immunogenicity of TA-HPV, a Recombinant Vaccinia Virus Expressing Modified Human Papillomavirus (HPV)-16 and HPV-18 E6 and E7 Genes, in Women with Progressive Cervical Cancer. *Clinical Cancer Research* 2002;8(12): 3676-3685.
- [128] Garcia-Hernandez E, Gonzalez, Sanchez J L, Andrade-Manzano A, Contreras M L, Padilla S, Guzman CC. Regression of Papilloma High-Grade Lesions (CIN 2 and CIN 3) is Stimulated by Therapeutic Vaccination with MVA E2 Recombinant Vaccine. *Cancer Gene Therapy* 2006;13(6): 592-597.
- [129] Kim JH, Kang TH, Noh KH, Bae HC, Kim SH, Yoo YD. Enhancement of Dendritic Cell-Based Vaccine Potency by Anti-Apoptotic siRNAs Targeting Key Pro-apoptotic Proteins in Cytotoxic CD8(+) T Cell-Mediated Cell Death. *Immunological Letters.* 2009;122(1): 58-67.
- [130] Ferrara A, Nonn M, Sehr P, Schreckenberger C, Pawlita M, Durst M. Dendritic Cell-Based Tumor Vaccine for Cervical Cancer II: Results of a Clinical Pilot Study in 15 Individual Patients. *Journal of Cancer Research and Clinical Oncology* 2003;129(9): 521-530.
- [131] Santin AD, Bellone S, Palmieri M, Zanolini A, Ravaggi A, Siegel ER. Human Papillomavirus Type 16 and 18 E7-Pulsed Dendritic Cell Vaccination of Stage IB or IIA

- Cervical Cancer Patients: a Phase I Escalating-Dose Trial. *Journal of Virology* 2008;82(4): 1968-1979.
- [132] National Taiwan University Hospital. Immunotherapy of Recurrent Cervical Cancers Using Dendritic Cells (DCs). 2009. Available from URL: <http://www.clinicaltrials.gov>.
- [133] Steller MA, Gurski KJ, Murakami M, Daniel RW, Shah KV, Celis E. Cell-Mediated Immunological Responses in Cervical and Vaginal Cancer Patients Immunized with a Lipidated Epitope of Human Papillomavirus Type 16 E7. *Clinical Cancer Research* 1998;4(9): 2103-2109.
- [134] Sharma RK, Elpek KG, Yolcu ES, Schabowsky RH, Zhao H, Bandura-Morgan L, Shirwan H. Costimulation as a Platform for the Development of Vaccines: a Peptide-Based Vaccine Containing a Novel Form of 4-1BB Ligand Eradicates Established Tumors. *Cancer Research* 2009;69(10): 4319-4326.
- [135] Barrios K, Celis E. TriVax-HPV: an Improved Peptide-Based Therapeutic Vaccination Strategy against Human Papillomavirus-Induced Cancers. *Cancer Immunology and Immunotherapy* 2012;61(8): 1307-1317.
- [136] Hallez S, Simon P, Maudoux F, Doyen J, Noel JC, Beliard A. Phase I/II Trial of Immunogenicity of a Human Papillomavirus (HPV) Type 16 E7 Protein-Based Vaccine in Women with Oncogenic HPV-Positive Cervical Intraepithelial Neoplasia. *Cancer Immunology and Immunotherapy* 2004;53(7): 642-650.
- [137] Frazer IH, Quinn M, Nicklin JL, Tan J, Perrin LC, Ng P. Phase 1 Study of HPV16-Specific Immunotherapy with E6E7 Fusion Protein and ISCOMATRIX Adjuvant in Women with Cervical Intraepithelial Neoplasia. *Vaccine* 2004;23(2): 172-181.
- [138] Einstein MH, Kadish AS, Burk RD, Kim MY, Wadler S, Streicher H. Heat Shock Fusion Protein-Based Immunotherapy for Treatment of Cervical Intraepithelial Neoplasia III. *Gynecologic Oncology*. 2007;106(3): 453-460.
- [139] Trimble CL, Peng S, Kos F, Gravitt P, Viscidi R, Sugar E. A Phase I Trial of a Human Papillomavirus DNA Vaccine for HPV16+ Cervical Intraepithelial Neoplasia 2/3. *Clinical Cancer Research* 2009;15(1): 361-367.
- [140] VGX Pharmaceuticals, Inc. Phase I of Human Papillomavirus (HPV) DNA Plasmid (VGX-3100)+ Electroporation for CIN 2 or 3, 2009. Available from URL: <http://www.clinicaltrials.gov>
- [141] Kim SW, Yang JS. Human Papillomavirus type 16 E5 Protein as a Therapeutic Target. *Yonsei Medical Journal* 2006;47(1): 1-14.
- [142] Liu DW, Tsao YP, Hsieh CH, Hsieh JT, Kung JT, Chiang CL, Huang SJ, Chen SL. Induction of CD8 T Cells by Vaccination with Recombinant Adenovirus Expressing Human Papillomavirus Type 16 E5 Gene Reduces Tumor Growth. *Journal of Virology* 2000;74(19): 9083-9089.
- [143] Chen YF, Lin CW, Tsao YP, Chen SL. Cytotoxic-T Lymphocyte human Papillomavirus type 16 E5 Peptide with CpG-Oligodeoxynucleotide Can Eliminate Tumor Growth in C57BL/6 Mice. *Journal of Virology* 2004;78: 1333-1343.
- [144] Mariz FC. Development of new biotechnology platforms for production of HPV vaccines: Approaches in the context of developing countries. *J Vaccines Vaccin* 2012;3:4.