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# The Role of Epigenetics in Cervical Cancer

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## Abstract

Cervical cancer is the fourth most common type of cancer among women worldwide resulting in 528,475 new cases and 268,224 deaths. The principal etiological factor of cervical cancer is the persistent infection with high-risk types of human papillomaviruses (HPV), however is not sufficient, other factors like age, smoking, oral contraceptives, and genetic background are implicated in the development of this neoplasia. Although the understanding of cervical carcinogenesis has been increasing in recent decades, the epigenetic modifications (DNA methylation, histone modification, miRNAs and long non-coding RNAs) and its contribution to the development of cervical cancer remain largely unknown. In the next chapter, we will recapitulate the described findings on the alteration of epigenetic factors that, together with the persistent infection of HPV, could contribute to the malignant and invasive phenotype in cervical cancer.

**Keywords:** HPV, DNA methylation, histone modification, ncRNAs, therapy

## 1. Introduction

Cervical cancer is the fourth most common type of cancer among women worldwide, resulting in 528,475 new cases per year with 268,224 deaths [1]. Cervical cancer represents 6.6% of all female cancers and nearly 90% of all deaths occur in both low- and middle-income countries, as the disease is detected in the advanced stages or when the treatment is inaccessible [2]. The principal etiological factor of cervical cancer is the persistent infection with high-risk types of human papillomaviruses (hr-HPV). In fact, the HPV prevalence among women with normal cytology worldwide was 11.7%. This estimate varies by geography being Sahara African regions (24%), Latin America and the Caribbean (16.2%), Eastern Europe (14.2%), and Southeast Asia (14%) the regions with the highest percentage of prevalence [3].

Most of hr-HPV premalignant lesions have a spontaneously viral clearance with a mean of 3 months in age-independent manner. Nonetheless, the cytological regression takes a longer time. This period depends in great manner on the grade of the lesion and if one or several hr-HPV are present. While mild and moderate/severe premalignant lesions with no HPV presence takes a mean of 5–6 months to recovery; mild, moderated, or severe premalignant lesions with the presence of

hr-HPV takes a mean of 17, 24, and 60 months, respectively [4, 5]. However, although hr-HPV persistent infection is necessary for the development of cervical cancer, the solely infection is not sufficient. The presence of factors like age [6, 7], smoking [8], oral contraceptives [9], alcohol usage [10], and host and viral genetic background are necessary to observe an accumulation of epithelial cell abnormalities like sustained proliferation and growth of new blood vessels. These abnormalities emerge due to genomic alteration, defects in the genome maintenance and repair, destabilization of the number of DNA copies, and/or somatic mutations. Then, the cells that harbor all these abnormalities can evolve progressively to a tumorigenic, and further, a malignant and invasive phenotype [11].

## **2. Papillomaviruses**

HPVs are DNA viruses that are able to infect the skin or mucosa of animal species. More than 200 human papillomavirus genotypes are known and have been categorized into phylogenetic genera as Alpha, Beta, Gamma, Mu, and Nu. The high-risk types of the Alpha genus are sexually transmitted being the types 16, 18, 52, 31, 58, 39, 51, and 56 the most common hr-HPV type found in women with apparent normal cytology. hr-HPV16 is the most frequently detected followed by hr-HPV18 and both are present in 70% of all the cervical cancers [12].

Papillomaviruses consist of a circular double-stranded DNA genome of approximately 8000 base pairs that harbor two main DNA structures: a long control region (LCR) which contains union sites for both, host cellular transcription factors and the viral proteins E1 and E2 that control viral replication and gene expression; and the open reading frames that codify to eight genes necessary for the maintenance and replication of the viral DNA. The high-risk alpha papillomaviruses present two well-characterized promoters: late promoter (LP or p670) which regulate gene expression of late proteins L1 and L2; and early promoter (PE or p97) which controls gene expression of early proteins E1, E2, E4, E5, E6, and E7. These genes are expressed by a complex pattern of mRNA splicing at different stages of the viral life cycle. The early and late viral proteins exert different function in the infected cell. E1 and E2 are involved in the viral genome replication, L1 and L2 orchestrate the virus assembly, and the E4, E5, E6, and E7 alter the replication machinery of the infected cell to facilitate the virus replication. Due to the target of the viral proteins E6 and E7 in the host cell, these proteins have been termed viral oncoproteins [13, 14].

The main interaction partner of HPV-E6 is the E3 ubiquitin ligase E6-associated protein (E6AP) which in turn targets the tumor suppressor p53 and proteins with a PDZ domain to proteasomal degradation to promote de-differentiation, impairing apoptosis induction, and eliminate cell cycle checkpoints of the infected cell [15–17]. HPV-E7 binds to multiple proteins of the Rb family members, such as pRb, p107, and p130 (collectively referred as pocket proteins) that is more extensively studied. hr-HPV E7 uses a short stretch of residues known as LXCXE motif and residues in its N-terminus interact and target degradation of the three Rb family members. The proteasome-mediated destruction of E7/Rb pocket proteins is mediated by the recruitment of Cullin 2 E3 ubiquitin ligase complex, allowing the infected cell to remain in a proliferative state [18–20]. It has been observed that a correlation between viral DNA integration to host cell genomic material and a higher expression of E6 and E7 viral protein, provides an advantage in the cellular growing and oncogenic progression by promoting cell proliferation, abrogating the cell cycle checkpoints, and causes genomic instability [21–23]. Since HPV is considered the principal risk factor in cervical cancer, it is also associated with other

cancer types like vulvar, vaginal, anal, penile, and oropharyngeal in females and males, the Advisory Committee on Immunization Practices (ACIP) recommend the routine vaccination with one of the three commercial available vaccines against HPV (9-valent, 4-valent, and 2-valent HPV vaccines, (HPVV)) in females and males at age 11 or 12 years and females aged 11–26 years and males aged 13 through 21 years not vaccinated previously. 2vHPVV contains HPV 16,18 virus-like particles; 4vHPVV contains HPV 6, 11, 16, and 18 virus-like particles; and 9vHPVV 6, 11, 16, 18, 31, 33, 45, 52, and 58 virus-like particles. These vaccines show a CIN prevention efficacy of 98% [24, 25]. Based in the above observations, these data highlight the importance of vaccination against HPVs since it seems like the expression of the HPV genome is the first step for development of pre-cancer lesions and a possible malignant progression. In this chapter, we review activities of E6 and E7 modulating epigenetics in cervical cancer and how these modifications could contribute to the development of this neoplasia.

Traditionally, cancer has been viewed as a multifactorial genetic disease that raise from an accumulation of mutations in tumor suppressor and/or oncogenes that cause loss or gain of function and an abnormal genetic expression. Although the understanding of cervical carcinogenesis has been increasing in recent decades, the epigenetic modifications (DNA methylation, histone modification and non-coding RNA (ncRNA)) and its contribution to the development of cervical cancer remain unknown. Nonetheless, in the past years, multiple epigenetic modifications have been associated with cancer initiation and proliferation [26]. The epigenetic are all the heritable changes in gene expression that are not due to changes in the nucleotide sequence of DNA. These modifications are established during embryonic development to bring cellular identity and are stably maintained during cellular replication in differentiated tissues. This is achieved by controlling the accessibility of transcription factors and by altering the capability of DNA packaging, having as result a temporal and spatial modulation in gene expression. Collectively, these modifications are referred as the epigenome. The epigenome comprises four main phenomena: Pos-translational histone modifications, DNA methylation, chromatin remodeling, and regulation by non-coding RNAs [26–28]. Recently, different works have been shown that hr-HPV E6 and E7 viral proteins have the capability of target key proteins which regulate epigenetic marks.

### **3. DNA methylation**

The DNA methylation is associated with gene silencing due the recruitment and/or disassociation of DNA-binding proteins that can act as repressor complexes or transcription factors which generate a transcriptional silencing. Moreover, the methylation is necessary for a correct embryonic development [15], genome stability [16], X chromosome inactivation [17, 18], genomic imprinting [19], and silence of retrotransposons [20]. In mammals, the predominant form of DNA methylation occurs by a covalent addition of a methyl group in the fifth carbon of cytosine residues that are preceded by guanine nucleotides (CpG dinucleotides) in both DNA strands. This methyl group comes from a universal donor called S-adenosyl-L-methionine (SAM) and the enzymatic reaction is controlled by 3 DNA methyltransferases named DNMT1, DNMT3A, and DNMT3B, and the enzymatically inactive proteins DNMT2 and DNMT3L [21, 22]. Nearly 80% of all the DNA CpG dinucleotides in somatic tissues are methylated and comprises satellite DNAs, repetitive elements like transposons, non-repetitive intergenic DNA, and exons of genes [23]. From this DNA elements, there are CpG dinucleotides that are non-methylated that can be detected in germ cells, early embryo, and in somatic tissues.

These CpG dinucleotides are concentrated in short DNA stretches with an average length from 500 to 2000 base pairs (bp) that are known as CpG Islands (CGIs) [24]. The main characteristics of the CGIs are an elevated G + C base concentration, low CpG depletion, absence of DNA methylation, and are preferentially located at 5' end of genes, occupying approximately 60% of human gene promoters [25–27].

In general, DNA methylation of CpG around the Transcription Start Site (TSS) is negatively correlated with gene expression, whereas a low DNA methylation around TSS and a high DNA methylation in the gene body are positively correlated with gene expression [28]. It has been reported that DNMT3A is overexpressed in HPV positive tumors and that DNMT1 overexpression leads to an increased overall DNA methylation and transformation of NIH 3 T3 cells [29, 30]. Also, it has been shown an increase in DNMT1 protein levels in low-grade CIN and in SCC in comparison with normal epithelium [31]. These observations positioned DNMT1 as a regulator of tumor progression. Interestingly, the analysis of genome wide methylation in squamous carcinoma (SCC) cell lines reveals that in SCC cells HPV positive harbors higher CpG methylation in repetitive regions and in genic and non-genic non-repetitive regions in comparison to SCC HPV negative cells [30]. This HPV-mediated DNA methylation increase can be explained by the modulation of E6 and E7 proteins over the expression and activity of the DNA methylation machinery that is described as follow.

The DNMT1 is known as maintenance methyltransferase. During the DNA replication, DNMT1 ensures that hemi-methylated CpG sites in the newly synthesized DNA maintain the methylation patterns accurately using as template for parental strand [32], whereas Dnmt3A and Dnmt3b mediate the de novo DNA methylation and establish the pattern of methylation in embryonic development [33]. The DNMT1 gene expression is controlled by the complex conformed by the tumor suppressor p53, transcription factor Specificity Protein 1 (SP1), and the Histone Deacetylases 1 and 6 (p53-SP1-HDAC1/6). This complex binds to SP1 binding sites near the DNMT1 promoter [34]. When present, E6 oncoprotein collaborates to increase the DNMT1 expression. In vitro assays shown that HPV16-E6 increases DNA methylation levels by stimulating expression and activity of DNMT1 by p53 suppression [35, 36]. As p53 is targeted to degradation by hr-HPV-E6 and E3 ubiquitin ligase E6-associated protein (E6AP) [37], the complex p53-SP1-HDAC1/6 could be disrupted increasing the levels of SP1 in the cell and leading to an SP1-mediated DNMT1 protein expression. Moreover, it has been shown that if SP1 protein levels increases, it is capable to target p53 to degradation by MDM2-mediated ubiquitination [34]. On the other hand, E7 oncoprotein binds directly to DNMT1 mediated by the C-terminal zinc-finger CR3 domain of E7, upregulating the methyltransferase activity and stabilizing the DNMT1 protein [38, 39]. This direct activation of DNMT1 by E7 could be potentiated in a positive feedback manner since the transcription of the gene is regulated by pRB/E2F1 [40]. Interestingly, Cicchini and colleagues shown that near E7-dependent hypermethylated clusters are an enrichment of EPAS1, FOXJ3, CDX2, IRF4, FOXF1, and GCR transcription factor binding motifs, suggesting that HPV16-E7 is capable to direct DNMT1 to silence gene promoters through an E7-transcription factor interaction [41]. Although it has been reported that the interaction of E7 with different transcription factors [42–44] and cells expressing hr-HPV viral DNA harbors a plethora of hypermethylated genes [30, 41, 45–54] (See **Table 1**), further experiments are needed to clarify this data.

The ability of HPV to maintain a persistent infection resides on mechanisms of immune host response evasion. The major histocompatibility complex (MHC-I)  $\alpha$ -subunit HLA-E is significantly downregulated by hypermethylation in a distant regulatory CpG island by HPV16-E7 suggesting that E7 alters immune cell

Gene	Reference	Gene	Reference
APC	[55]	MGMT	[45, 48, 49]
C8ORF4	[56]	MRC2	[54]
C13ORF18	[51]	MT1G	[57]
CADM1	[50]	NKX2-8	[54]
CCNA1	[58, 59]	NMES1	[56]
CCND2	[60]	NPTX-1	[54]
CDH1	[46, 56, 61]	p16	[46, 48]
CDH13	[60]	P73	[62]
CDKN2A	[49]	PHACTR3	[54]
CLIC3	[54]	PRDM14	[54]
CNNA1	[51, 58, 59]	PTEN	[63]
CREB3LI	[54]	RAR-62	[64]
CxCL 14	[65]	RARB	[60]
DAPK	[45, 46, 49, 60]	RASSF1A	[66]
DDK3	[53]	RASSF2	[52]
E-cadherin	[67]	RRAD	[56]
H-cadherin	[67]	SFRP1	[56]
EPB41L3	[52]	SFRP2	[53]
FAM19A4	[54]	SFRP4	[53]
FHIT	[47, 49]	SFRP5	[53]
HLA-E	[41]	SLCA4	[54]
FLJ36166	[56]	SOST	[54]
FN1	[56]	SOX17	[53]
GPNMB	[56]	SPARC	[56]
HSPA2	[56]	SSX4	[56]
hTERT	[45, 48, 49, 51]	TFPI2	[56]
INK4A	[48]	TIMP-3	[46]
LFNG	[54]	TNFSF13	[54]
LHX1	[54]	TSCL1	[68]
MAL	[50]	TWIST1	[51, 60]
		WDFY3	[54]

**Table 1.**  
*Cervical cancer genes hypermethylated reported in literature.*

recognition during early stages of persistent infection [41]. On the other hand, CxCL 14 is a chemokine that functions as a potent angiogenesis inhibitor and a chemotactic factor for dendritic and natural killer cells [69, 70]. It has been seen that E7 downregulates the chemokine CXCL14 by a direct hypermethylation of its promoter. If the CxCL14 expression is restored, an increase of the presence of natural killer and CD8+ T cells in tumor-draining lymph nodes is observed [65]. HPV also inhibit the ability of Langerhans cells (antigen presenting cells) to infiltrate into the virus infected area by reducing the E-cadherin expression on infected keratinocytes cell membrane [71]. It has been demonstrated that in oral tongue,

breast, and prostate cell lines as well as breast and prostate tumors that Enhancer of Zeste Homolog 2 (EZH2), Embryonic Ectoderm Development (EED), and Suppressor of Zeste 12 (ZUS12), components of the Polycomb Repressive Complex 2 (PRC2) along with Histone Deacetylase 1 (HDAC1) are responsible of E-cadherin silencing by Histone 3 lysine 27 trimethylation (H3K27me3) on E-cadherin promoter [72, 73]. Since it has been reported that HPV16-E6 and E7 induce a decrease in the transcription levels of E-cadherin gene without targeting E-cadherin to proteasome degradation or methylation of the E-cadherin promoter [36, 39], this PRC2 silencing mechanism could be the responsible of E7-mediated E-cadherin-downregulation due E7 can induce EZH2 expression via liberation of E2F transcription factors from the inhibitory activity of pRB, p107, and p130 [74]. EZH2 increase expression could arise the formation of PRC2 that, in turn, can recruit and hyperactivate type 1 Histone Deacetylases (HDAC-1) leading to histone deacetylation and a subsequent trimethylation in H3K27 at the E-cadherin promoter silencing its expression [75, 76]. In addition, it has been shown that hr-HPV16 E7 can block HDAC-HIF-1 $\alpha$  interaction [77] leading to a possible increase in HDAC free levels that can interact with PRC2. Moreover, HPV16/18 E6 and E7 oncoproteins increase the expression of thymopoietin pseudogene 2 (TMPOP2; lncRNA-EBIC) a long non-coding RNA that is repressed in cis by p53 transcription factor (see below). This lncRNA-EBIC can interact with EZH2 generating a TMPOP2-EZH2 complex that has been postulated as a PRC2-recruit facilitator to E-cadherin promoter region silencing these gene [78, 79].

Although the hypermethylation gene status is predominant in the hr-HPV host cell genome, there are works that demonstrate a hypomethylation in promotor genes (See **Table 2**). Yin et al., analyzed the expression and promoter methylation status of STK31 gene in cell lines and cervical tumors expressing hr-HPV. They found an increased expression and a hypomethylation of STK31 CpG islands in HPV16/18-positive HeLa, SiHa, and CaSki cervical cancer cell lines and HPV16/18-positive pre-malignant lesion Cervical Intraepithelial Neoplasia grade 3 (CIN3) and Cervical Cancer (CC) biopsies compared with HPV-negative C33A and HT-3 cervical cancer cell lines and HPV-negative CIN3 and CC. In addition, the authors reported that STK31 promotor were hypermethylated in all normal, CIN1, and CIN2 biopsies analyzed. However, STK3 promoter were hypomethylated in all CIN3 and CC biopsies analyzed being found more often hypomethylated in CIN3 than in CC [82]. Other genes found to be hypomethylated were Rap guanidine Nucleotide Exchange Factor (RAPGEF1) and Cancer Antigen Gene (CAGE). Samuelsson and colleagues shown that 48% of cervical squamous carcinomas analyzed present no methylation in CGI near RAPGEF1 promoter and hypomethylation on a CGI present in the first intron of these gene [80]. Lee and colleagues analyzed the methylation status of CAGE promotor gene in 40 cervical cancer patients finding that 87.5% of the samples were hypomethylated in comparison of control non-neoplastic tissues [81].

Gene	Reference
RAPGEF1	[80]
CAGE	[81]
STK31	[82]
COL17A1	[83]
Ribosomal DNA	[84]

**Table 2.**  
*Cervical cancer genes hypomethylated reported in literature.*

Interestingly, HPV16 DNA is an efficient target for DNA methylation by host cell DNA methylation machinery. The viral DNA is organized into nucleosomes in equal form that eukaryote DNA [85, 86]. This viral DNA organization can modulate the viral gene expression by DNA methylation and histone modifications. The E2 viral protein is the master regulator of E6 and E7 expression by binding into four conserved E2-binding sites (E2BS) that are located in the LCR close to DNA binding sites of several cellular transcription factors like TATA-binding protein, AP-1, Sp1, GPS2/AMF-1, TopoBP1, CDP, and YY1. These E2BS have a consensus DNA sequence 5'-ACCG(n)4CGGT-3' upstream of the p97 early promoter. The E2 viral protein can activate or repress viral transcription in a dose dependent manner. At low concentrations E2 binds to E2BS4 due its great affinity, leaving the E6 promoter active. When E2 rises, the low affinity binding sites E2BS1 and E2BS2 are occupied by E2 blocking the binding of transcription factors and the recruitment of transcriptional repressors at the E6 promoter, preventing E6 and E7 transcription [87–91]. In addition, E2 is able to bind the double bromodomain protein Brd4, through of its C-terminal region and the bromodomain-containing region BDR4 recruits E2 viral protein by its N-terminal and C-terminal DNA binding domain region to E2BS-4, thus preventing the Transcription Factor II D (TFIID) and polymerase II interaction with TATA box and E6 promoter region, respectively [92]. The E2-BDR4 complex also represses the interaction between BDR4 and the Positive Transcription Elongation Factor b (P-TEFb) which is necessary to E6 and E7 expression [93]. In this way, the loss of regulation of the E2 viral protein deregulate the expression of E6 and E7 viral proteins, which can in turn contribute to further malignant transformation. HPV genome integration usually occurs in the E1 and E2 ORF regions generating a loss of E2 negative expression control allowing unregulated transcription of E6 and E7 viral genes [90, 94]. The viral integration has been shown to occur in two different ways: as a single genome and a head-to-tail multiple tandem repeats correlating positively the amount of CpG methylation with the number of integrated viral genome copies [95–97]. If multiple viral DNA copies are integrated in host genome, only one copy is transcriptionally active due a extensively methylation of the other integrated genome viral copies [95]. Otherwise, has been shown in vitro that E2 viral protein E2BSs binding capability is impaired by CpG methylation being more prevalent E2BS1 site methylated. These E2BSs methylation in the HPV16 LCR trigger the overexpression of E6 and E7 viral proteins [95, 97–99]. Moreover, the grade of methylation in E2BSs and in LCR varies in great manner depending of the differentiated status of the host cell, being highly methylated in less well differentiated cells and hypomethylated in LCR of viral genomes in more highly differentiated epithelial cells, correlating with the E6 and E7 course expression in infecting cells [100]. In addition to disruption of E2 ORF, the methylation of specific CpG present in hr-HPV LCR leads to an increase expression of E6 and E7 viral genes even if E2 viral protein still expressing in the host cell. All these observations underscore the combined mechanisms conducted by E6 and E7 in the methylation and hypomethylation to achieve an optimum environment for viral replication.

#### **4. Pos-translational histone modifications**

It is importantly to note that the E6 and E7 capability of altering gene expression can occur by interaction with a subset of chromatin-modifying enzymes that are flanking target genes. In higher eukaryotes and double-stranded DNA viruses, the DNA is tightly wrapping around a heterogeneous multi-unit structure termed nucleosome. The nucleosome is the core unit of chromatin which is 146-bp length



DNA wound around octameric of the four highly conserved histone proteins (H3, H4, H2A, and H2B). Each nucleosome is linked one to other by a stretch of DNA called DNA linker with a length of 40–55 bp. The chromatin gives DNA structure and regulates the gene transcription via post-translational modifications (PTM). These PTMs are modifications such as acetylation, methylation, phosphorylation, ubiquitination, sumoylation, glycosylation, homocysteinylation, crotonylation, propionylation, and butyrylation in the amino-terminal and carboxy-terminal tail of histones that are mediated by diverse histone modifying enzymes. These PTMs regulate gene expression by affecting the nucleosome stability and structure [101, 102].

The E6 and E7 viral proteins can alter the chromatin structure by association and/or modifying the enzymatic activity and/or altering the expression of chromatin-remodeling enzymes. HPV16-E7 modulates the immune host response downregulating a subset of proteins by methylation. Viral nucleic acids are sensed by a pathogen recognition receptor (PRR) called toll-like receptor 9 (TLR9) that are expressed in keratinocytes. This receptor allows the recognition of unmethylated double-stranded DNA CpG motifs present in the HPV DNA and initiate a signaling cascade that leads to the production of type I Interferon (INF) and proinflammatory cytokines which in turn activates host immune defenses against the infection. Nonetheless, *in vitro* experiments have been shown that HPV16-E7 suppress TLR9 transcription by inducing the formation of a repressive chromatin modification complex which is formed by ER $\alpha$ , HDAC1, JARID1B, and NF- $\kappa$ B p50-p65 at specific NF- $\kappa$ B element (site B) of TLR9 promoter. Recruited by ER $\alpha$ , JARID1B prevents the trimethylation of histone 3 at lysin 4 (H3K4me3) and HDAC-1 prevents the acetylation of histone 4 (AcH4) from the site B until the transcription start site of the TLR9 promoter in C33A cells with HPV16 [103]. However, two different reports observed that TLR9 expression was only expressed in fully differentiated keratinocytes and in different layers of HPV-positive cervical epithelia neoplasia and that TLR9 expression is primary intracellular in cervical epithelium [104, 105]. Another study conducted by Canella and collaborators observed that TLR9 expression under presence of low-risk or high-risk HPV and an increase in the TLR9 protein expression in patients with persistent HPV infection. The authors argue that the discrepancies in the TLR9 expression in HPV infected cells reside in a balance between the strength of TLR9 inhibition by HPV and the subject capability to drive proper TLR9 activation [106]. However, further studies are needed to elucidate this data discrepancy.

HPV16-E7 also interferes with downstream signaling of TLRs. It has been seen that E7 interacts *in vivo* and *in vitro* with the Interferon Regulatory Factor-1 (IRF-1). IRF-1 is a transcription factor how belong to a family of 9 DNA-binding factors are called from IRF-1 to IRF-9. IRF-1 recognizes a central 11–13 nucleotide core region denominated INF stimulated response elements (ISREs) [107]. These regulatory elements are present in the promoters of INF- $\beta$  and some INF-inducible genes [108]. HPV16-E7 interacts directly with its CR1/2 domains and the carboxyl-terminal transactivation domain of IRF-1, eliminating its transactivation function of IRF-1 both *in vitro* and *in vivo*. Moreover, the Nucleosome remodeling and deacetylase (NuRD) complex could be implicated since HPV16-E7 interacts directly with Mi2 $\beta$  (a subunit of the NuRD complex) via C-terminal zinc-finger CR3 domain leading to a chromatin deacetylation and silencing IRF-1-dependent transcription suppressing cellular immune response due viral infection [109, 110].

E6 and E7 viral proteins can alter the activity of histone acetyltransferases (HAT) and histone deacetylases (HDAC). NF- $\kappa$ B is a transcription factor composed of homodimers or heterodimers complexes of five subunits named p50, p52, p65/Rel A, c-Rel, and Rel B; being p50/p65 the most common dimer. To achieve a

correct NF- $\kappa$ B transcription, it is necessary the recruitment and interaction with different transcriptional coactivators like CREB binding protein (CBP), p300, Steroid Receptor-Coactivator-1 (SRC-1), or Nuclear receptor CoActivator-1 (NCoA-1) [111]. This interaction is mediated by Protein Kinase A (PKA) phosphorylation in p65/Rel A serine 276 residue unmasking the CPB-interaction domain present in p65/Rel A. This phosphorylation generates a conformational change that permits a bivalent interaction; first with CBP KIX domain (450–679 aa) and 276 phosphorylated p65-serine and last with CBP region comprised by 313–450 aa CBP and p65 region flanked by 477–504 aa [112]. The transcription of multiple p53-regulated genes is mediated by cyclic-AMP-regulated enhancer (CRE) transcription factor (CREB) and the HAT CBP, p300, and HMT PRMT1, CARM1, and SET7 coactivators that modulate the methylation and acetylation of histones surrounding p53 target genes [113, 114]. The complex CREB–CBP can bind to specific transcription factors where recruit and bind with histone binding factor RbAp48. This CREB–CBP–RbAp48 complex allows the interaction and subsequent CBP/p300 acetylation of target genes histones leading to a chromatin structure rearrange and recruitment of transcription machinery [115–120]. Moreover, An and coworkers demonstrated that *in vivo* and *in vitro* PRMT1 and CARM1 interacts directly with p53 trough N-terminal (1–43 aa) and C-terminal (370–393 aa), respectively. Also, they shown that are a cooperatively functions in p53 transcription by p300, PRMT1, and CARM1 coactivators for an optimal p53 transcription activity, being necessary the ordered recruitment to p53-responsive genes: first PRMT1 is recruited and methylate H4R3, then a p300 accumulation and H4 acetylation, and last a subsequent CARM1 accumulation and H3R17 methylation [114]. Like phosphorylation, it has been shown *in vitro* and *in vivo* that p53 can be activated and stabilized against ubiquitin-mediated degradation by SET7-mediated mono-methylation in residue 372 (p53-K372me1) and, presumably, a subsequent CBP/p300-mediated acetylation [121, 122]. The CBP/p300-p53 complex can interact with multiple p300 and p53 domains. It has been shown that p300 domains like N-terminal Taz1 domain (CH1 domain; 302–451 aa), KIX domain (588–683 aa), C-terminal Taz2 domain (CH3 domain; 1514–1737 aa), and nuclear receptor coactivator binding domain (NCBD; 2059–2117 aa) can interact with p53 TAD (1–61 aa) and DNA-binding Core Domain (90–160 aa) [123–127]. This CBP/p300-p53 interaction promotes p53 C-terminal domain (363–393 aa) acetylation leading to increase in p53-DNA binding and transcription activity *in vivo* and *in vitro* [123, 124, 128, 129].

Lee and coworkers demonstrated that p53 TAD multisite phosphorylation enhances p53 affinity for Taz1, Taz2, and KIX domains of CBP leading to a graded p53 response to genotoxic stress [130]. On other side, *in vivo* and *in vitro* experiments shown that the second zinc finger present in C-terminal region of HPV 16/18-E6 (aa 100–107) interact with CBP/p300 via its Transcriptional Adapter Motif (TRAM), a 19-aa sequence present in CBP II domain, competing with the CBP/p300-p53 interaction [131]. Also, has been shown that E6 interacts with p300 CH1 domain (340–413 aa) and NCBD domain (1970–2220 aa) generating a E6-p53-p300 complex without E6AP participation. This trimeric complex inhibits both p300-mediated acetylation of p53 and nucleosomal core histones abrogating the p53-dependent transcription activated by CBP/p300. In addition to a p53-E6-E6AP, *in vitro* and *in vivo*, HPV18-E6 promotes p53 degradation by direct association and inhibition of SET7 methyltransferase activity that stabilizes p53 by mono-methylation in K372 residue. Whereas not all p53 is promoted to degradation due loss of K372me1, HPV18-E6 can abolishes the p53-dependent remnant gene transcription by direct interaction and downregulation of coactivators CARM1, PRMT1, and SET7 methyltransferase activities, generating a reduced p53 DNA binding and loss of p53 gene expression [122]. Notably, DNMT1 is associated and mono-

methyated in K142 residue (DNMT1-K142) by SET7 causing its degradation [132]. Thus, it is possible that the presence of E6 abrogates the SET7-dependent degradation of DNMT1 increasing the free protein levels that can interact with E7 viral protein, generating an increased activity earlier described of DNMT1-E7 protein complex. Further experiments needed to demonstrate this hypothesis.

Also, hr-HPV 16-E6 disrupt the NF- $\kappa$ B-dependent transactivation by binding competition on N-terminus CH1 domain and C-terminus of CBP that are recognition sites of RelA/p65 and SCR-1, respectively. Furthermore, HPV16-E7 also suppresses the NF- $\kappa$ B-dependent transactivation. The N terminal (1–51 aa) region of E7 viral protein interact both in vitro and in vivo with TAZ2 domain of transcriptional coactivator CBP/p300. Notably, this interaction increases if HPV16-E7 CKII site (Ser31 and Ser32) is phosphorylated [129, 133–137]. hr-HPV16-E7 also can bind to P/CAF HAT domain (352–658 aa) via E7-leucine 67 residue diminishing P/CAF acetyltransferase activity [135].

## 5. HPV RNA targets

It has been described that, in humans, less than 3% of genome encodes to protein-coding exons while more than 85% of genome is transcribed into non-coding RNAs (ncRNAs) [138, 139]. These ncRNAs can be classified accordingly by their size as short or long ncRNAs. Micro RNAs (miRNAs) are a group of small non-coding single-strand RNA of 19–24 nucleotides that play key roles in differentiation and development by post-transcriptional regulation of cellular genes. Their main function is to repress the expression of target mRNA by cleavage or translational silencing depending of the degree of miRNA sequence complementation with the 3'-UTR of target mRNAs [140]. The HPV viral proteins can target different RNA species modifying their expression (See **Tables 3–5**). For example, HPV16 E2 and E6 viral proteins interact with RNA molecules and reduce the pre-RNA splice efficiency. The N-terminal trans activation domain and the hinge region of HPV16-E2 (1–220 aa and 221–259 aa respectively) and the central region of HPV16-E6 (42–102 aa) are the responsibly of splicing suppression; whereas the E2 C-terminal DNA-binding domain (260–365 aa) and the E6 C-terminal Nuclear Localization Signal (NLS3) domain (115–124 aa) are the protein portions responsible for protein-RNA interaction. Moreover, HPV16-E2 can interact with splicing factors SRp30, SRp40, SRp55, and SRp75 and HPV16-E6 interacts with SRp30, SRp55, and SRp75 via C-terminal of both viral proteins [173]. miRNA-23b is located in the intron 14 of the host gene C9ORF3 on chromosome 9. This miRNA regulates c-MET gene which mediates cellular apoptosis via AKT signaling pathway. When HPV16-E6 is present, C9ORF3 and the intronic miRNA-23b is downregulated by DNMT1-mediated CGI hypermethylation located 1 kb upstream from the transcription start site of C9ORF3 gene [174].

The miR-375 has been shown to regulate the HPV viral gene expression in vitro and in vivo. miR-375 can downregulates E6 and E7 viral transcription due the presence of two putative binding sites present in the E7 region (677–698 aa; 687–708 aa) and three in the E1 region (1236–1258 aa; 1259–1280 aa; 1862–1884 aa) of the HPV genome. Also, this miRNA in vivo and in vitro can bind directly the 3'UTR of E6AP and the transcription factor SP1 diminishing E6AP and SP1 mRNA and protein. As a result of E6AP and SP1 proteins degradation mediated by miR-375, an increase in p21, p53, and Rb proteins can be observed [175–177]. However, in vitro assays demonstrated that HPV16-E6 can hypermethylate DNMT1-mediated miR-375 promoter region [178] downregulating miR-375 and leading an increase in SP1 transcription factor levels, thereby, contributing to DNMT1-positive loop feedback

Gene up-regulated	Reference	Gene down-regulated	Reference
AC007879.7	[144]	MEG3	[153,154]
CCAT	[160, 161]	MIR205HG	[144]
CCEPR	[163]	OIS1	[155]
CCHE1	[142, 143]	PVT1	[156]
FAM83H	[144]	RP3-510D11.2	[144]
GAS5	[144]	RP6-65G23.3	[144]
GS1-600G8.5	[144]	RP11-479G22.8	[144]
H19	[144]	RP13.463N16.6	[144]
HOTAIR	[149]	RSU1P2	[157]
HOXC-As5	[144]	SFTA1P	[144]
LINC00963	[144]	SNHG15	[144]
LINC01057	[144]	SPRY4-IT1	[159]
lncRNA LET	[151]	TMPOP2 (lncRNA-EBIC)	[79]
MAFG-AS1	[144]	XIST	[162]
MALAT1	[152]	XLOC_010588	[164]

**Table 3.**  
*lncRNAs reported up- and down-regulated in literature.*

described early. Moreover, miR-124 and miR-375 mediated a reciprocal regulation with long non-coding RNA MALAT1. If miR-375 is overexpressed a significant reduction in MALAT1 expression is observed. This regulation could be by direct interaction between miR-375 and MALAT1 due miR-375 has two putative MALAT1 binding sites whereas MALAT1 harbors two putative binding sites with miR-124 [169, 178]. Future experiments are necessary to elucidate which factors influence the downregulation of both, cellular and viral gene expression and the molecular factors are involved in HPV E6 and E7 interaction with these miRNAs and MALAT1.

Otherwise, the long non-coding RNAs (lncRNAs) are transcripts of more than 200 nucleotides in length. These RNAs possess structural characteristics of messenger RNAs (mRNAs) like that are transcribed by RNA Polymerase II, spliced, harbor a poly adenylated tail, and a 5'-capping. lncRNAs can modulate transcription, alternative splicing, mRNA stability, mRNA translation and chromatin remodeling by bind to RNA, DNA, or a subset of proteins. Interestingly, Khalil and colleagues showed that the mammalian genome encodes nearly 4500 lncRNAs and approximately 24% of these lncRNAs interact with chromatin-modifying proteins like the repressive complex PRC2, CoREST, and SCMx [179]. Due their role in distinct cellular processes, HPV viral proteins can modulate multiple host's lncRNAs [140].

As described earlier, the long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) was associated with cell proliferation and invasion in HPV positive cervical cancer cells [152, 169, 180]. Also, in CaSki cell line, the transfection of MALAT1 increases the expression of cyclin D1, cyclin E and cyclin-dependent kinase 6 (CDK6). When HPV16 E6 and E7 are downregulated, MALAT1 expression is downregulated too, indicating that these viral proteins are involved in the MALAT1 expression [152]. However, further studies are needed to elucidate the mechanism of MALAT1 regulation by HPV.

Barr and colleagues identify a subset of lncRNAs upper and downregulated in primary human foreskin keratinocytes which express HPV16-E6 viral protein. The

Gene up-regulated	Reference	Gene up-regulated	Reference	Gene up-regulated	Reference
let-7e	[165]	miR-181c	[165, 172]	miR-30b	[165]
let-7i	[165]	miR-182	[170]	miR-30d	[165]
miR-106a	[167, 168]	miR-183	[170]	miR-30e	[165]
miR-106b	[168, 171, 172]	miR-185	[168]	miR-326	[165]
miR-10	[165]	miR-186	[165]	miR-339-5p	[168]
miR-10b	[168]	miR-187	[165]	miR-340	[165]
miR-1224-5p	[168]	miR-192	[172]	miR-342	[165]
miR-124	[172]	miR-194	[165]	miR-34a	[165]
miR-126	[165]	miR-195	[165]	miR-34c	[165]
miR-127	[165]	miR-196a	[141]	miR-374	[165]
miR-129	[165]	miR-199a	[165]	miR-449a	[172]
miR-130a	[165]	miR-199b	[165]	miR-449b	[172]
miR-130b	[165, 168]	miR-199s	[165]	miR-512-3p	[172]
miR-132	[141, 165]	miR-19a	[165]	miR-517a	[172]
miR-133a	[165]	miR-20	[165]	miR-517c	[172]
miR-133b	[165]	miR-200a	[165]	miR-518f	[172]
miR-134	[165]	miR-200c	[170]	miR-542-3p	[172]
miR-135a	[165]	miR-205	[170]	miR-545	[172]
miR-135b	[165, 172]	miR-20a	[158, 167]	miR-625	[168]
miR-139	[165]	miR-20b	[168]	miR-7g	[165]
miR-140	[165]	miR-21	[145, 165, 168, 171]	miR-886-5p	[167]
miR-141	[172]	miR-210	[170]	miR-9	[165]
miR-142-3p	[165]	miR-213	[165]	miR-92a	[167]
miR-142-5p	[165]	miR-214	[165]	miR-93	[167, 168]
miR-145	[165]	miR-215	[165]	miR-941	[168]
miR-146	[165]	miR-218	[165]	miR-98	[165]
miR-146a	[166]	miR-223	[166]		
miR-146b-5p	[168]	miR-224	[167]		
miR-148a	[141]	miR-25	[165]		
miR-150	[165]	miR-26a	[165]		
miR-151	[165]	miR-26b	[165]		
miR-155	[166, 167, 168]	miR-28	[165]		
miR-15	[165, 166, 168]	miR-29a	[165]		
miR-15b	[166, 167, 171]	miR-29b	[165]		
miR-16	[167, 171, 172]	miR-301	[165]		
miR-17	[168]	miR-301b	[172]		
miR-181a	[165]	miR-302b	[141]		
miR-181b	[165]	miR-30a-3p	[165]		

**Table 4.**  
*miRNAs reported up-regulated in literature.*

authors found that FAM83H-AS1 is overexpressed by HPV16-E6 viral protein mediated by p300, and its inhibition decrease proliferation, migration, and resistance to apoptosis in vitro, whereas in pre-malignant and cervical cancer tissues the

Gene	Reference	Gene	Reference
let-7a-c	[145]	miR-218	[99, 166, 167]
let-7b	[145]	miR-23b	[145, 166]
let-7c	[145]	miR-26a	[141]
miR-100	[168]	miR-29a	[167]
miR-101	[166]	miR-328	[168]
miR-10b	[167]	miR-34a	[166]
miR-124	[169]	miR-368	[170]
miR-125b	[167, 168, 171]	miR-370	[171]
miR-126	[167, 170]	miR-375	[167, 168]
miR-139-3p	[168]	miR-379	[168]
miR-139-5p	[168]	miR-381	[168]
miR-143	[166, 170]	miR-424	[166, 167]
miR-145	[166, 168, 170]	miR-433	[172]
miR-149	[168]	miR-494	[171]
miR-188	[171]	miR-497	[168, 170]
miR-193b	[171]	miR-513	[141]
miR-195	[167, 168, 170]	miR-572	[171]
miR-196b	[145]	miR-574-3p	[168]
miR-199a	[141]	miR-575	[171]
miR-199a-5p	[168]	miR-617	[168]
miR-199b-5p	[168]	miR-638	[171]
miR-203	[171]	miR-99a	[167, 168]

**Table 5.**  
*miRNAs reported down-regulated in literature.*

high expression of FAM83H-AS1 correlates with worse overall survival compared with normal cervix samples [144].

The lncRNA HOX Transcript Antisense Intergenic RNA (HOTAIR) can binds to and recruits the PRC2 to repress transcription of multiple gene loci in trans. HOTAIR expression is downregulated in earlier stages of cervical cancer. However, in HPV16 positive cervical carcinomas and in HPV positive cell lines which harbor a higher HPV16-E7 protein expression, the lncRNA HOTAIR is upregulated correlating with high HPV16-E7 expression level. Moreover, HPV16-E7 interacts with HOTAIR. This interaction could impair the formation of the PCR2 complex generating diminish of H3K27me3 repression mark and thus increasing the expression of a large number of genes [149, 181, 182]. Interestingly, the HPV16-E7-HOTAIR interaction generates an autoregulatory loop between HOTAIR, miR-331-3p and Neuropilin 2 (NRP2). It has been shown that HOTAIR is a competitive endogenous RNA (ceRNA) showing a sponge effect over miR-331-3p and that miR-331-3p directly regulates NRP2. So, when is present, HPV16-E7 interacts and diminishes HOTAIR expression generating an increase of miR-331-3p levels due the lack of HOTAIR sponge effect over miR-331-3p. The miR-331-3p induce a decrease of NRP2 levels by binding through 3'UTR of NRP. Being NRP2 a HPV16-E7 transcription regulator, the downregulation of NRP2 protein levels lead to a diminished HPV16-E7 protein levels too, generating a regulatory loop [183, 184].

As described early, thymopoietin pseudogene 2 (TMPOP2, lncRNA-EBIC) is a lncRNA that interact with EZH2 to repress E-cadherin gene expression. Interestingly, this lncRNA regulates the expression of HPV viral genes in cervical cancer cells. Several miRNAs, like miR-375 and miR-139, can target to degradation the HPV16/18 E6 and E7 mRNA. However, lncRNA-EBIC also acts as a ceRNA, sequestering miR-375 and miR-139 increasing the E6 and E7 viral gene expression. Moreover, the upregulation of E6 and E7 by lncRNA-EBIC lead to p53 degradation which is a transcriptional repressor of lncRNA-EBIC, generating a positive loop feedback [79].

The lncRNA LET [151], GAS5 [146], and MEG3 [153, 154] expression is downregulated in cervical cancer tissues and is associated with poor prognosis, malignant status, lymph node metastasis, invasion, and shorter overall survival. The expression of MEG3 leads to an increase in cell apoptosis, increased levels of p53 and cleaved caspase 3 in cervical cancer cells. Also, this lncRNA can regulate the expression levels of miR-21-5p [153, 154].

On the contrary, the lnc Ras Suppressor Protein 1 Pseudogene 2 (RSU1P2) expression is upregulated in cervical cancer tissues and promotes proliferation, invasion, and migration of cervical cancer cell lines. Moreover, in vitro and in vivo assays demonstrated that RSU1P2 acts as ceRNA binding directly to and downregulating let7a expression, leading to an increase of Let-7a target genes as IGF1R, N-myc, and EphA4. Interestingly, let-7a can target the 3-UTR of N-Myc inhibiting its mRNA and protein production, whereas N-Myc can bind to RSU1P2 promoter region and increase its transcription. Therefore, N-Myc can forms a positive loop feedback with RSU1P2 increasing its oncogenic activity [157]. If any HPV viral protein can modulate this pathway is currently unknown.

The lncRNA Plasmacytoma Variant Translocation 1 (PVT1) expression is upregulated in cervical cancer tissues and correlates positively with poor overall survival. If PVT1 expression is inhibit a decrease in cellular proliferation, migration, and invasion is observed whereas apoptosis and cisplatin toxicity increase in cervical cancer cell lines [156].

There are numerous lncRNAs that have been poorly investigated in their molecular mechanism in HPV-infected cervical carcinoma cells. However, some studies described the correlations between lncRNAs expression and clinical characteristics of cervical cancer patients. For example, the lncRNA Colon Cancer-Associated Transcript 2 (CCAT2) [160, 161], SPRY4-IT1 [159], and CCHE1 [142] are highly expressed and positively associated with cell proliferation and survival of cervical cancer cells as well malignant status and poor prognosis of cervical cancer patients. CCHE1 high expression promotes cell proliferation of cervical cancer cells. Interestingly, CCHE1 physically interacts with Proliferating Cell Nuclear Antigen (PCNA) mRNA increasing the PCNA gene expression. This PCNA expression is necessary for the proliferation effect of CCHE1 [143].

## **6. Therapeutic approaches**

The balance alteration of oncogenes and tumor-suppressor genes creates an advantage to cancer cells. Many of these alterations are due epigenetic alterations such DNA methylation, histone modification, and/or non-coding RNAs expression/repression. However, this cancer cells advantage can serve also as therapeutic targets to counterattack cancer pathogenesis and progression. Currently, there are some studies describing drugs that alter these epigenetic changes present in cervical cancer cells.

A study employs a peripheral vasodilator drug and DNA methylation inhibitor called Hydralazine. The authors employed hydralazine at 40  $\mu\text{mol/L}$  for 72 h and

they observed a restoration of APC gene expression in HeLa and CaSki cervical cancer cells. This gene re-expression was due to APC promoter region demethylation [55]. In 2005, Zambrano and colleagues mounted a phase 1 study of hydralazine employing different dosages (from 25 mg/8 h to 50 mg/8 h) for a 10 days period. They found that employing any hydralazine concentration tested, eight tumor suppressors genes were demethylate and re-expressed in untreated cervical cancer patients without affecting global DNA methylation [185].

Another compound capable to restore gene expression of tumor suppressor genes hypermethylated is Trichosanthin (TCS). TCS is a 237 aa type I ribosome-inactivating protein extracted from the root tubers of the Chinese medical herb *Trichocanthes kirilowi*. Huang and colleagues reported increases mRNA and protein levels of APC and TSLC1 due demethylation in the CpG islands in the promoter region in HeLa and CaSki cervical cancer cells treated with 20, 40 and 80 µg/ml for 48 h presumable mediated by DNMT1 since its mRNA, protein levels, and enzyme activity decreases following the treatment in a dose-dependent manner [68]. However, until these data shown a likely useful as a demethylating agent for treatment, this work does not report the toxicity effects over non-transformed cell lines.

In another study, hydralazine was proved in combination with the HDAC inhibitor valproate acid. After 5 days of Hydralazine at 10 µM and magnesium Valproate at 1 mM treatment, SiHa, CasKi, and HeLa cervical cancer cells lead to a small increase HPV gene expression due demethylation and acetylated H4 enrichment at 5' region of LCR. However, a p53 gene expression and protein levels were increased after treatment whit Hydralazine, Valproate, or in combination in CasKi, HeLa, and SiHa cell lines being p53 stability likely due 373 and 382 lysine p53 hyperacetylation that protects from E6-mediated degradation. Also, the hydralazine/valproate phase II trial with treatment of Hydralazine at 182 or 83 mg and magnesium Valproate at 40 mg/kg shown that E6 and E7 transcripts remains unchanged in primary tumors of patients with cervical cancer, suggesting that epigenetic therapy cannot facilitate increase of viral oncogene activation [186].

On the other hand, apicidin, an inhibitor of histone deacetylases, induces downregulation of DNMT1 and increase p21WAF1/Cip1 expression in HeLa cervical cancer cell line. The Apicidin-mediated DNMT1 downregulation is achieved by a significant H3 and H4 hypoacetylation, depletion of H3K4me3 gene transcription mark, and enriched H3K9me3 and H3K27me3 repressive marks in the nucleosomes on DNMT1 transcriptional initiation site. Moreover, Apicidin treatment lead to a decreased Pol II presence on the transcription initiation site and the recruitment of co-repressors pRB and HDAC1 and dissociation of activators P/CAF and HAT from the E2F consensus-binding site on the DNMT1 promoter site. However, HeLa cells treated solely with Apicidin does not induce apoptosis of HeLa cells in comparison of DNMT1 knock down which cause an apoptotic effect, indicating that other targets are needed to achieve Apicidin therapeutic effect [187].

Quercetin a flavonoid found in fruits and vegetables also have epigenetics effects, it has been reported that quercetin induces attenuating lipid peroxidation, platelet aggregation, capillary permeability, anti-proliferative, anti-migratory, and proapoptotic effect in HeLa cervical carcinoma cells [188]. Employing doses of 25 and 50 µM, Quercetin can inhibit the activity of DNMT1, HDACs, H3K9 HMT activity, in a dose-dependent manner. Using the same Quercetin concentrations was observed a decreased methylation percentage and increase APC, CDH1, CDH13, DAPK1, FHTI, GSTP1, MGMT, MLH1, PTEN, RARB, RASSF1, SOC51, TIMP3, and VHL expression and a global DNA methylation in a dose-dependent manner. Also, Quercetin modulates the expression of several enzymes and chromatin modifiers like HDAC2, HDAC1, DNMT1, HDAC3, HAT1, DNMT3B, HDAC7, HDAC6, HDAC11, DNMT3A, and HDAC5 in a dose-dependent manner [189]. Interestingly,



those therapeutic approaches described here were tested employing cervical cancer models. However, it would be interesting to explore the effectiveness of these approaches on HPV-infected anus and oral models where HPV is associated with malignant transformation [150, 190–192].

## 7. Conclusions

Here we describe the epigenetic regulation mechanisms observed when hr-HPV is present in cervical cancer. The viral oncoproteins expression from hr-HPV induce genetic and epigenetic changes in the cells that contribute to malignant transformation and development of cervical cancer. These modifications could be used as biomarkers and new therapeutic molecules that could help in the treatment of cervical cancer.

## Conflict of interest

The authors declare no conflict of interest.

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