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

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the
TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

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

The Role of Epigenetics in Cervical Cancer

Yair Alfaro-Mora, Luis A. Herrera,
Rodrigo Cáceres-Gutiérrez, Marco A. Andonegui-Elguera,
Guadalupe Dominguez-Gómez and José Díaz-Chávez

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 Southeaster 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-asociated 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 whit an overage 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-asociated 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) α-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 (PCR2) 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-1α interaction [77] leading to a possible increase in HDAC free levels that can interact with PCR2. 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 where 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 promotor 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). This PTM are modifications such 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 PTM 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 witch is formed by ERα, HDAC1, JARID1B, and NF-kB p50-p65 at specific NF-kB element (site B) of TLR9 promoter. Recruited by ERα, 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-β 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 Mi2β (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-κB 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 dimmer. To achieve a

correct NF-κB 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 CoAtivator-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-

methylated 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-κB-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-κB-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′-caping. 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	[166–168]	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 resis-
tance 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 µmol/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 where tested employing cervical cancer models. However, it would be interesting 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.

Author details

Yair Alfaro-Mora[1], Luis A. Herrera[2], Rodrigo Cáceres-Gutiérrez[2],
Marco A. Andonegui-Elguera[2], Guadalupe Dominguez-Gómez[2]
and José Díaz-Chávez[2*]

1 Department of Genetics and Molecular Biology, Center for Research and Advanced Studies (CINVESTAV-IPN), Mexico City, México

2 Biomedical Research Unit in Cancer, Biomedical Research Institute, UNAM/ National Cancer Institute (INCan), Mexico City, México

*Address all correspondence to: josediaz030178@hotmail.com

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018;**68**(6):394-424

[2] WHO. Cervical Cancer. Geneva: World Health Organization; 2018. Available from: http://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/

[3] Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjose S. Cervical human papillomavirus prevalence in 5 continents: Meta-analysis of 1 million women with normal cytological findings. The Journal of Infectious Diseases. 2010;**202**(12): 1789-1799

[4] Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, Rozendaal L, Voorhorst FJ, Bezemer PD, et al. Cytological regression and clearance of high-risk human papillomavirus in women with an abnormal cervical smear. Lancet. 2001;**358**(9295): 1782-1783

[5] Schiffman M, Doorbar J, Wentzensen N, de Sanjose S, Fakhry C, Monk BJ, et al. Carcinogenic human papillomavirus infection. Nature Reviews. Disease Primers. 2016;**2**:16086

[6] Pirtea L, Grigoras D, Matusz P, Pirtea M, Moleriu L, Tudor A, et al. Age and HPV type as risk factors for HPV persistence after loop excision in patients with high grade cervical lesions: An observational study. BMC Surgery. 2016;**16**(1):70

[7] Castle PE, Schiffman M, Herrero R, Hildesheim A, Rodriguez AC, Bratti MC, et al. A prospective study of age trends in cervical human papillomavirus acquisition and persistence in Guanacaste, Costa Rica. The Journal of infectious diseases. 2005; **191**(11):1808-1816

[8] International Collaboration of Epidemiological Studies of Cervical Cancer, Appleby P, Beral V, Berrington de Gonzalez A, Colin D, Franceschi S, et al. Carcinoma of the cervix and tobacco smoking: Collaborative reanalysis of individual data on 13,541 women with carcinoma of the cervix and 23,017 women without carcinoma of the cervix from 23 epidemiological studies. International Journal of Cancer. 2006;**118**(6):1481-1495

[9] Marks M, Gravitt PE, Gupta SB, Liaw KL, Tadesse A, Kim E, et al. Combined oral contraceptive use increases HPV persistence but not new HPV detection in a cohort of women from Thailand. The Journal of Infectious Diseases. 2011;**204**(10): 1505-1513

[10] Oh HY, Kim MK, Seo S, Lee DO, Chung YK, Lim MC, et al. Alcohol consumption and persistent infection of high-risk human papillomavirus. Epidemiology and Infection. 2015; **143**(7):1442-1450

[11] Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;**144**(5):646-674

[12] Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: A meta-analysis update. International Journal of Cancer. 2007; **121**(3):621-632

[13] Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;**30** (Suppl 5):F55-F70

[14] Wise-Draper TM, Wells SI. Papillomavirus E6 and E7 proteins and their cellular targets. Frontiers in Bioscience: A Journal and Virtual Library. 2008;**13**:1003-1017

[15] Li C, Fan Y, Li G, Xu X, Duan J, Li R, et al. DNA methylation reprogramming of functional elements during mammalian embryonic development. Cell Discovery. 2018;**4**:41

[16] Gopalakrishnan S, Sullivan BA, Trazzi S, Della Valle G, Robertson KD. DNMT3B interacts with constitutive centromere protein CENP-C to modulate DNA methylation and the histone code at centromeric regions. Human Molecular Genetics. 2009;**18**(17):3178-3193

[17] Sharp AJ, Stathaki E, Migliavacca E, Brahmachary M, Montgomery SB, Dupre Y, et al. DNA methylation profiles of human active and inactive X chromosomes. Genome Research. 2011;**21**(10):1592-1600

[18] Sado T, Okano M, Li E, Sasaki H. De novo DNA methylation is dispensable for the initiation and propagation of X chromosome inactivation. Development. 2004;**131**(5):975-982

[19] Biniszkiewicz D, Gribnau J, Ramsahoye B, Gaudet F, Eggan K, Humpherys D, et al. Dnmt1 overexpression causes genomic hypermethylation, loss of imprinting, and embryonic lethality. Molecular and Cellular Biology. 2002;**22**(7):2124-2135

[20] Xie M, Hong C, Zhang B, Lowdon RF, Xing X, Li D, et al. DNA hypomethylation within specific transposable element families associates with tissue-specific enhancer landscape. Nature Genetics. 2013;**45**(7):836-841

[21] Goll MG, Bestor TH. Eukaryotic cytosine methyltransferases. Annual Review of Biochemistry. 2005;**74**:481-514

[22] Lyko F. The DNA methyltransferase family: A versatile toolkit for epigenetic regulation. Nature Reviews. Genetics. 2018;**19**(2):81-92

[23] Li E, Zhang Y. DNA methylation in mammals. Cold Spring Harbor Perspectives in Biology. 2014;**6**(5): a019133

[24] Jeziorska DM, Murray RJS, De Gobbi M, Gaentzsch R, Garrick D, Ayyub H, et al. DNA methylation of intragenic CpG islands depends on their transcriptional activity during differentiation and disease. Proceedings of the National Academy of Sciences of the United States of America. 2017; **114**(36):E7526-E7E35

[25] Takai D, Jones PA. Comprehensive analysis of CpG islands in human chromosomes 21 and 22. Proceedings of the National Academy of Sciences of the United States of America. 2002;**99**(6): 3740-3745

[26] Gardiner-Garden M, Frommer M. CpG islands in vertebrate genomes. Journal of Molecular Biology. 1987; **196**(2):261-282

[27] Bird A, Taggart M, Frommer M, Miller OJ, Macleod D. A fraction of the mouse genome that is derived from islands of nonmethylated, CpG-rich DNA. Cell. 1985;**40**(1):91-99

[28] Laurent L, Wong E, Li G, Huynh T, Tsirigos A, Ong CT, et al. Dynamic changes in the human methylome during differentiation. Genome Research. 2010;**20**(3):320-331

[29] Wu J, Issa JP, Herman J, Bassett DE Jr, Nelkin BD, Baylin SB. Expression of an exogenous eukaryotic DNA methyltransferase gene induces transformation of NIH 3T3 cells. Proceedings of the National Academy of Sciences of the United States of America. 1993;**90**(19):8891-8895

[30] Sartor MA, Dolinoy DC, Jones TR, Colacino JA, Prince ME, Carey TE, et al. Genome-wide methylation and expression differences in HPV(+) and HPV(−) squamous cell carcinoma cell lines are consistent with divergent mechanisms of carcinogenesis. Epigenetics. 2011;**6**(6):777-787

[31] Sawada M, Kanai Y, Arai E, Ushijima S, Ojima H, Hirohashi S. Increased expression of DNA methyltransferase 1 (DNMT1) protein in uterine cervix squamous cell carcinoma and its precursor lesion. Cancer Letters. 2007;**251**(2):211-219

[32] Leonhardt H, Page AW, Weier HU, Bestor TH. A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. Cell. 1992;**71**(5):865-873

[33] Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell. 1999;**99**(3):247-257

[34] Lin RK, Wu CY, Chang JW, Juan LJ, Hsu HS, Chen CY, et al. Dysregulation of p53/Sp1 control leads to DNA methyltransferase-1 overexpression in lung cancer. Cancer Research. 2010; **70**(14):5807-5817

[35] Au Yeung CL, Tsang WP, Tsang TY, Co NN, Yau PL, Kwok TT. HPV-16 E6 upregulation of DNMT1 through repression of tumor suppressor p53. Oncology Reports. 2010;**24**(6): 1599-1604

[36] D'Costa ZJ, Jolly C, Androphy EJ, Mercer A, Matthews CM, Hibma MH. Transcriptional repression of E-cadherin by human papillomavirus type 16 E6. PLoS One. 2012;**7**(11):e48954

[37] Thomas M, Pim D, Banks L. Human papillomavirus E6 protein interactions. In: McCance DJ, editor. Perspectives in Medical Virology. Vol. 8. Netherlands: Elsevier; 2002. pp. 71-99

[38] Burgers WA, Blanchon L, Pradhan S, de Launoit Y, Kouzarides T, Fuks F. Viral oncoproteins target the DNA methyltransferases. Oncogene. 2007;**26**(11):1650-1655

[39] Laurson J, Khan S, Chung R, Cross K, Raj K. Epigenetic repression of E-cadherin by human papillomavirus 16 E7 protein. Carcinogenesis. 2010;**31**(5): 918-926

[40] McCabe MT, Davis JN, Day ML. Regulation of DNA methyltransferase 1 by the pRb/E2F1 pathway. Cancer Research. 2005;**65**(9):3624-3632

[41] Cicchini L, Blumhagen RZ, Westrich JA, Myers ME, Warren CJ, Siska C, et al. High-risk human papillomavirus E7 alters host DNA methylome and represses HLA-E expression in human keratinocytes. Scientific Reports. 2017;**7**(1):3633

[42] Antinore MJ, Birrer MJ, Patel D, Nader L, McCance DJ. The human papillomavirus type 16 E7 gene product interacts with and trans-activates the AP1 family of transcription factors. The EMBO journal. 1996;**15**(8):1950-1960

[43] Luscher-Firzlaff JM, Westendorf JM, Zwicker J, Burkhardt H, Henriksson M, Muller R, et al. Interaction of the fork head domain transcription factor MPP2 with the human papilloma virus 16 E7 protein: Enhancement of transformation and transactivation. Oncogene. 1999; **18**(41):5620-5630

[44] 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. The Journal of Biological Chemistry. 2002; **277**(4):2923-2930

[45] Iliopoulos D, Oikonomou P, Messinis I, Tsezou A. Correlation of promoter hypermethylation in hTERT, DAPK and MGMT genes with cervical oncogenesis progression. Oncology Reports. 2009;**22**(1):199-204

[46] Jeong DH, Youm MY, Kim YN, Lee KB, Sung MS, Yoon HK, et al. Promoter methylation of p16, DAPK, CDH1, and TIMP-3 genes in cervical cancer: Correlation with clinicopathologic characteristics. International Journal of Gynecological Cancer: Official Journal of the International Gynecological Cancer Society. 2006;**16**(3):1234-1240

[47] Ki KD, Lee SK, Tong SY, Lee JM, Song DH, Chi SG. Role of 5'-CpG island hypermethylation of the FHIT gene in cervical carcinoma. Journal of Gynecologic Oncology. 2008;**19**(2): 117-122

[48] Lin Z, Gao M, Zhang X, Kim YS, Lee ES, Kim HK, et al. The hypermethylation and protein expression of p16 INK4A and DNA repair gene O6-methylguanine-DNA methyltransferase in various uterine cervical lesions. Journal of Cancer Research and Clinical Oncology. 2005; **131**(6):364-370

[49] Banzai C, Nishino K, Quan J, Yoshihara K, Sekine M, Yahata T, et al. Promoter methylation of DAPK1, FHIT, MGMT, and CDKN2A genes in cervical carcinoma. International Journal of Clinical Oncology. 2014;**19**(1):127-132

[50] Overmeer RM, Louwers JA, Meijer CJ, van Kemenade FJ, Hesselink AT, Daalmeijer NF, et al. Combined CADM1 and MAL promoter methylation analysis to detect (pre-) malignant cervical lesions in high-risk HPV-positive women. International Journal of Cancer. 2011;**129**(9):2218-2225

[51] Milutin Gasperov N, Sabol I, Planinic P, Grubisic G, Fistonic I, Corusic A, et al. Methylated host cell gene promoters and human papillomavirus type 16 and 18 predicting cervical lesions and cancer. PLoS One. 2015;**10**(6):e0129452

[52] Guerrero-Setas D, Perez-Janices N, Blanco-Fernandez L, Ojer A, Cambra K, Berdasco M, et al. RASSF2 hypermethylation is present and related to shorter survival in squamous cervical cancer. Modern Pathology: An Official Journal of the United States and Canadian Academy of Pathology, Inc. 2013;**26**(8):1111-1122

[53] van der Meide WF, Snellenberg S, Meijer CJ, Baalbergen A, Helmerhorst TJ, van der Sluis WB, et al. Promoter methylation analysis of WNT/beta-catenin signaling pathway regulators to detect adenocarcinoma or its precursor lesion of the cervix. Gynecologic Oncology. 2011;**123**(1): 116-122

[54] Steenbergen RD, Ongenaert M, Snellenberg S, Trooskens G, van der Meide WF, Pandey D, et al. Methylation-specific digital karyotyping of HPV16E6E7-expressing human keratinocytes identifies novel methylation events in cervical carcinogenesis. The Journal of Pathology. 2013;**231**(1):53-62

[55] Song Y, Zhang C. Hydralazine inhibits human cervical cancer cell growth in vitro in association with APC demethylation and re-expression. Cancer Chemotherapy and Pharmacology. 2009;**63**(4):605-613

[56] Sova P, Feng Q, Geiss G, Wood T, Strauss R, Rudolf V, et al. Discovery of novel methylation biomarkers in cervical carcinoma by global demethylation and microarray analysis. Cancer Epidemiology, Biomarkers and Prevention: A Publication of the American Association for Cancer Research, cosponsored by the American

Society of Preventive Oncology. 2006;
15(1):114-123

[57] Li L, Xu C, Long J, Shen D, Zhou W, Zhou Q, et al. E6 and E7 gene silencing results in decreased methylation of tumor suppressor genes and induces phenotype transformation of human cervical carcinoma cell lines. Oncotarget. 2015;**6**(27):23930-23943

[58] Chalertpet K, Pakdeechaidan W, Patel V, Mutirangura A, Yanatatsaneejit P. Human papillomavirus type 16 E7 oncoprotein mediates CCNA1 promoter methylation. Cancer Science. 2015;**106**(10):1333-1340

[59] Zhang Y, Chen FQ, Sun YH, Zhou SY, Li TY, Chen R. Effects of DNMT1 silencing on malignant phenotype and methylated gene expression in cervical cancer cells. Journal of Experimental and Clinical Cancer Research. 2011;**30**:98

[60] Feng Q, Balasubramanian A, Hawes SE, Toure P, Sow PS, Dem A, et al. Detection of hypermethylated genes in women with and without cervical neoplasia. Journal of the National Cancer Institute. 2005;**97**(4): 273-282

[61] Holubekova V, Mendelova A, Grendar M, Mersakova S, Kapustova I, Jasek K, et al. Methylation pattern of CDH1 promoter and its association with CDH1 gene expression in cytological cervical specimens. Oncology Letters. 2016;**12**(4):2613-2621

[62] Jha S, Vande Pol S, Banerjee NS, Dutta AB, Chow LT, Dutta A. Destabilization of TIP60 by human papillomavirus E6 results in attenuation of TIP60-dependent transcriptional regulation and apoptotic pathway. Molecular Cell. 2010;**38**(5):700-711

[63] Yang HJ, Liu VW, Wang Y, Tsang PC, Ngan HY. Differential DNA

methylation profiles in gynecological cancers and correlation with clinico-pathological data. BMC Cancer. 2006;**6**: 212

[64] Liu L, Zhang J, Bates S, Li JJ, Peehl DM, Rhim JS, et al. A methylation profile of in vitro immortalized human cell lines. International Journal of Oncology. 2005;**26**(1):275-285

[65] Cicchini L, Westrich JA, Xu T, Vermeer DW, Berger JN, Clambey ET, et al. Suppression of antitumor immune responses by human papillomavirus through epigenetic downregulation of CXCL14. MBio. 2016;**7**(3)

[66] Cohen Y, Singer G, Lavie O, Dong SM, Beller U, Sidransky D. The RASSF1A tumor suppressor gene is commonly inactivated in adenocarcinoma of the uterine cervix. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research. 2003;**9**(8):2981-2984

[67] Widschwendter A, Ivarsson L, Blassnig A, Muller HM, Fiegl H, Wiedemair A, et al. CDH1 and CDH13 methylation in serum is an independent prognostic marker in cervical cancer patients. International Journal of Cancer. 2004;**109**(2):163-166

[68] Huang Y, Song H, Hu H, Cui L, You C, Huang L. Trichosanthin inhibits DNA methyltransferase and restores methylation-silenced gene expression in human cervical cancer cells. Molecular Medicine Reports. 2012;**6**(4):872-878

[69] Starnes T, Rasila KK, Robertson MJ, Brahmi Z, Dahl R, Christopherson K, et al. The chemokine CXCL14 (BRAK) stimulates activated NK cell migration: Implications for the downregulation of CXCL14 in malignancy. Experimental Hematology. 2006;**34**(8):1101-1105

[70] Shellenberger TD, Wang M, Gujrati M, Jayakumar A, Strieter RM, Burdick MD, et al. BRAK/CXCL14 is a

potent inhibitor of angiogenesis and a chemotactic factor for immature dendritic cells. Cancer Research. 2004; **64**(22):8262-8270

[71] Matthews K, Leong CM, Baxter L, Inglis E, Yun K, Backstrom BT, et al. Depletion of Langerhans cells in human papillomavirus type 16-infected skin is associated with E6-mediated down regulation of E-cadherin. Journal of Virology. 2003;**77**(15):8378-8385

[72] Cao Q, Yu J, Dhanasekaran SM, Kim JH, Mani RS, Tomlins SA, et al. Repression of E-cadherin by the polycomb group protein EZH2 in cancer. Oncogene. 2008;**27**(58):7274-7284

[73] Wang C, Liu X, Chen Z, Huang H, Jin Y, Kolokythas A, et al. Polycomb group protein EZH2-mediated E-cadherin repression promotes metastasis of oral tongue squamous cell carcinoma. Molecular Carcinogenesis. 2013;**52**(3): 229-236

[74] Holland D, Hoppe-Seyler K, Schuller B, Lohrey C, Maroldt J, Durst M, et al. Activation of the enhancer of zeste homologue 2 gene by the human papillomavirus E7 oncoprotein. Cancer Research. 2008;**68**(23):9964-9972

[75] van der Vlag J, Otte AP. Transcriptional repression mediated by the human polycomb-group protein EED involves histone deacetylation. Nature Genetics. 1999;**23**(4):474-478

[76] Kleer CG, Cao Q, Varambally S, Shen R, Ota I, Tomlins SA, et al. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. Proceedings of the National Academy of Sciences of the United States of America. 2003; **100**(20):11606-11611

[77] Bodily JM, Mehta KP, Laimins LA. Human papillomavirus E7 enhances

hypoxia-inducible factor 1-mediated transcription by inhibiting binding of histone deacetylases. Cancer Research. 2011;**71**(3):1187-1195

[78] Sun NX, Ye C, Zhao Q, Zhang Q, Xu C, Wang SB, et al. Long noncoding RNA-EBIC promotes tumor cell invasion by binding to EZH2 and repressing E-cadherin in cervical cancer. PLoS One. 2014;**9**(7):e100340

[79] He H, Liu X, Liu Y, Zhang M, Lai Y, Hao Y, et al. Human papillomavirus E6/E7 and long noncoding RNA TMPOP2 mutually upregulated gene expression in cervical cancer cells. Journal of Virology. 2019;**93**(8)

[80] Samuelsson J, Alonso S, Ruiz-Larroya T, Cheung TH, Wong YF, Perucho M. Frequent somatic demethylation of RAPGEF1/C3G intronic sequences in gastrointestinal and gynecological cancer. International Journal of Oncology. 2011;**38**(6): 1575-1577

[81] Lee TS, Kim JW, Kang GH, Park NH, Song YS, Kang SB, et al. DNA hypomethylation of CAGE promotors in squamous cell carcinoma of uterine cervix. Annals of the New York Academy of Sciences. 2006;**1091**: 218-224

[82] Yin FF, Wang N, Bi XN, Yu X, Xu XH, Wang YL, et al. Serine/threonine kinases 31(STK31) may be a novel cellular target gene for the HPV16 oncogene E7 with potential as a DNA hypomethylation biomarker in cervical cancer. Virology Journal. 2016;**13**:60

[83] Thangavelu PU, Krenacs T, Dray E, Duijf PH. In epithelial cancers, aberrant COL17A1 promoter methylation predicts its misexpression and increased invasion. Clinical Epigenetics. 2016;**8**:120

[84] Zhou H, Wang Y, Lv Q, Zhang J, Wang Q, Gao F, et al. Overexpression of

ribosomal RNA in the development of human cervical cancer is associated with rDNA promoter hypomethylation. PLoS One. 2016;**11**(10):e0163340

[85] Stunkel W, Bernard HU. The chromatin structure of the long control region of human papillomavirus type 16 represses viral oncoprotein expression. Journal of Virology. 1999;**73**(3): 1918-1930

[86] Favre M, Breitburd F, Croissant O, Orth G. Chromatin-like structures obtained after alkaline disruption of bovine and human papillomaviruses. Journal of Virology. 1977;**21**(3): 1205-1209

[87] Thierry F, Yaniv M. The BPV1-E2 trans-acting protein can be either an activator or a repressor of the HPV18 regulatory region. The EMBO Journal. 1987;**6**(11):3391-3397

[88] Steger G, Corbach S. Dose-dependent regulation of the early promoter of human papillomavirus type 18 by the viral E2 protein. Journal of Virology. 1997;**71**(1):50-58

[89] Romanczuk H, Thierry F, Howley PM. Mutational analysis of cis elements involved in E2 modulation of human papillomavirus type 16 P97 and type 18 P105 promoters. Journal of Virology. 1990;**64**(6):2849-2859

[90] Schwarz E, Freese UK, Gissmann L, Mayer W, Roggenbuck B, Stremlau A, et al. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. Nature. 1985; **314**(6006):111-114

[91] Demeret C, Desaintes C, Yaniv M, Thierry F. Different mechanisms contribute to the E2-mediated transcriptional repression of human papillomavirus type 18 viral oncogenes. Journal of Virology. 1997;**71**(12): 9343-9349

[92] Wu SY, Lee AY, Hou SY, Kemper JK, Erdjument-Bromage H, Tempst P, et al. Brd4 links chromatin targeting to HPV transcriptional silencing. Genes and Development. 2006;**20**(17):2383-2396

[93] Yan J, Li Q, Lievens S, Tavernier J, You J. Abrogation of the Brd4-positive transcription elongation factor B complex by papillomavirus E2 protein contributes to viral oncogene repression. Journal of Virology. 2010; **84**(1):76-87

[94] Romanczuk H, Howley PM. Disruption of either the E1 or the E2 regulatory gene of human papillomavirus type 16 increases viral immortalization capacity. Proceedings of the National Academy of Sciences of the United States of America. 1992; **89**(7):3159-3163

[95] Chaiwongkot A, Vinokurova S, Pientong C, Ekalaksananan T, Kongyingyoes B, Kleebkaow P, et al. Differential methylation of E2 binding sites in episomal and integrated HPV 16 genomes in preinvasive and invasive cervical lesions. International Journal of Cancer. 2013;**132**(9):2087-2094

[96] McBride AA, Warburton A. The role of integration in oncogenic progression of HPV-associated cancers. PLoS Pathogens. 2017;**13**(4): e1006211

[97] Vinokurova S, von Knebel Doeberitz M. Differential methylation of the HPV 16 upstream regulatory region during epithelial differentiation and neoplastic transformation. PLoS One. 2011;**6**(9):e24451

[98] Bhattacharjee B, Sengupta S. CpG methylation of HPV 16 LCR at E2 binding site proximal to P97 is associated with cervical cancer in presence of intact E2. Virology. 2006; **354**(2):280-285

[99] Thain A, Jenkins O, Clarke AR, Gaston K. CpG methylation directly inhibits binding of the human papillomavirus type 16 E2 protein to specific DNA sequences. Journal of Virology. 1996;**70**(10):7233-7235

[100] Kim K, Garner-Hamrick PA, Fisher C, Lee D, Lambert PF. Methylation patterns of papillomavirus DNA, its influence on E2 function, and implications in viral infection. Journal of Virology. 2003;**77**(23):12450-12459

[101] McAnena P, Brown JA, Kerin MJ. Circulating nucleosomes and nucleosome modifications as biomarkers in cancer. Cancers. 2017;**9**(1)

[102] Chen Y, Sprung R, Tang Y, Ball H, Sangras B, Kim SC, et al. Lysine propionylation and butyrylation are novel post-translational modifications in histones. Molecular and Cellular Proteomics—MCP. 2007;**6**(5):812-819

[103] Hasan UA, Zannetti C, Parroche P, Goutagny N, Malfroy M, Roblot G, et al. The human papillomavirus type 16 E7 oncoprotein induces a transcriptional repressor complex on the toll-like receptor 9 promoter. The Journal of Experimental Medicine. 2013;**210**(7):1369-1387

[104] Karim R, Meyers C, Backendorf C, Ludigs K, Offringa R, van Ommen GJ, et al. Human papillomavirus deregulates the response of a cellular network comprising of chemotactic and proinflammatory genes. PLoS One. 2011;**6**(3):e17848

[105] Andersen JM, Al-Khairy D, Ingalls RR. Innate immunity at the mucosal surface: Role of toll-like receptor 3 and toll-like receptor 9 in cervical epithelial cell responses to microbial pathogens. Biology of Reproduction. 2006;**74**(5):824-831

[106] Cannella F, Pierangeli A, Scagnolari C, Cacciotti G, Tranquilli G, Stentella P, et al. TLR9 is expressed in human papillomavirus-positive cervical cells and is overexpressed in persistent infections. Immunobiology. 2015;**220**(3):363-368

[107] Tanaka N, Kawakami T, Taniguchi T. Recognition DNA sequences of interferon regulatory factor 1 (IRF-1) and IRF-2, regulators of cell growth and the interferon system. Molecular and Cellular Biology. 1993;**13**(8):4531-4538

[108] Yanai H, Negishi H, Taniguchi T. The IRF family of transcription factors: Inception, impact and implications in oncogenesis. Oncoimmunology. 2012;**1**(8):1376-1386

[109] Brehm A, Nielsen SJ, Miska EA, McCance DJ, Reid JL, Bannister AJ, et al. The E7 oncoprotein associates with Mi2 and histone deacetylase activity to promote cell growth. The EMBO Journal. 1999;**18**(9):2449-2458

[110] 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. The Journal of Biological Chemistry. 2000;**275**(10):6764-6769

[111] Sheppard KA, Rose DW, Haque ZK, Kurokawa R, McInerney E, Westin S, et al. Transcriptional activation by NF-kappaB requires multiple coactivators. Molecular and Cellular Biology. 1999;**19**(9):6367-6378

[112] Zhong H, Voll RE, Ghosh S. Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. Molecular Cell. 1998;**1**(5):661-671

[113] Giebler HA, Lemasson I, Nyborg JK. p53 recruitment of CREB binding protein mediated through phosphorylated CREB: A novel pathway

of tumor suppressor regulation. Molecular and Cellular Biology. 2000; **20**(13):4849-4858

[114] An W, Kim J, Roeder RG. Ordered cooperative functions of PRMT1, p300, and CARM1 in transcriptional activation by p53. Cell. 2004;**117**(6):735-748

[115] Jin Q, Yu LR, Wang L, Zhang Z, Kasper LH, Lee JE, et al. Distinct roles of GCN5/PCAF-mediated H3K9ac and CBP/p300-mediated H3K18/27ac in nuclear receptor transactivation. The EMBO Journal. 2011;**30**(2):249-262

[116] Hennig AK, Peng GH, Chen S. Transcription coactivators p300 and CBP are necessary for photoreceptor-specific chromatin organization and gene expression. PLoS One. 2013;**8**(7):e69721

[117] Raisner R, Kharbanda S, Jin L, Jeng E, Chan E, Merchant M, et al. Enhancer activity requires CBP/P300 bromodomain-dependent histone H3K27 acetylation. Cell Reports. 2018; **24**(7):1722-1729

[118] Ramos YF, Hestand MS, Verlaan M, Krabbendam E, Ariyurek Y, van Galen M, et al. Genome-wide assessment of differential roles for p300 and CBP in transcription regulation. Nucleic Acids Research. 2010;**38**(16):5396-5408

[119] Zhang Q, Vo N, Goodman RH. Histone binding protein RbAp48 interacts with a complex of CREB binding protein and phosphorylated CREB. Molecular and Cellular Biology. 2000;**20**(14):4970-4978

[120] Ito T, Ikehara T, Nakagawa T, Kraus WL, Muramatsu M. p300-mediated acetylation facilitates the transfer of histone H2A-H2B dimers from nucleosomes to a histone chaperone. Genes and Development. 2000;**14**(15):1899-1907

[121] Ito A, Lai CH, Zhao X, Saito S, Hamilton MH, Appella E, et al.

p300/CBP-mediated p53 acetylation is commonly induced by p53-activating agents and inhibited by MDM2. The EMBO Journal. 2001;**20**(6):1331-1340

[122] Hsu CH, Peng KL, Jhang HC, Lin CH, Wu SY, Chiang CM, et al. The HPV E6 oncoprotein targets histone methyltransferases for modulating specific gene transcription. Oncogene. 2012;**31**(18):2335-2349

[123] Avantaggiati ML, Ogryzko V, Gardner K, Giordano A, Levine AS, Kelly K. Recruitment of p300/CBP in p53-dependent signal pathways. Cell. 1997;**89**(7):1175-1184

[124] Gu W, Shi XL, Roeder RG. Synergistic activation of transcription by CBP and p53. Nature. 1997; **387**(6635):819-823

[125] Van Orden K, Giebler HA, Lemasson I, Gonzales M, Nyborg JK. Binding of p53 to the KIX domain of CREB binding protein. A potential link to human T-cell leukemia virus, type I-associated leukemogenesis. The Journal of Biological Chemistry. 1999;**274**(37): 26321-26328

[126] Grossman SR, Perez M, Kung AL, Joseph M, Mansur C, Xiao ZX, et al. p300/MDM2 complexes participate in MDM2-mediated p53 degradation. Molecular Cell. 1998;**2**(4):405-415

[127] Teufel DP, Freund SM, Bycroft M, Fersht AR. Four domains of p300 each bind tightly to a sequence spanning both transactivation subdomains of p53. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(17):7009-7014

[128] Gu W, Roeder RG. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell. 1997;**90**(4):595-606

[129] Thomas MC, Chiang CM. E6 oncoprotein represses p53-dependent

gene activation via inhibition of protein acetylation independently of inducing p53 degradation. Molecular Cell. 2005;**17**(2):251-264

[130] Lee CW, Ferreon JC, Ferreon AC, Arai M, Wright PE. Graded enhancement of p53 binding to CREB-binding protein (CBP) by multisite phosphorylation. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**(45): 19290-19295

[131] Zimmermann H, Degenkolbe R, Bernard HU, O'Connor MJ. The human papillomavirus type 16 E6 oncoprotein can down-regulate p53 activity by targeting the transcriptional coactivator CBP/p300. Journal of Virology. 1999; **73**(8):6209-6219

[132] Esteve PO, Chin HG, Benner J, Feehery GR, Samaranayake M, Horwitz GA, et al. Regulation of DNMT1 stability through SET7-mediated lysine methylation in mammalian cells. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**(13):5076-5081

[133] 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. The EMBO Journal. 1999;**18**(18): 5061-5072

[134] Huang SM, McCance DJ. Down regulation of the interleukin-8 promoter by human papillomavirus type 16 E6 and E7 through effects on CREB binding protein/p300 and P/CAF. Journal of Virology. 2002;**76**(17): 8710-8721

[135] Avvakumov N, Torchia J, Mymryk JS. Interaction of the HPV E7 proteins with the pCAF acetyltransferase. Oncogene. 2003; **22**(25):3833-3841

[136] Bernat A, Massimi P, Banks L. Complementation of a p300/CBP defective-binding mutant of adenovirus E1a by human papillomavirus E6 proteins. The Journal of General Virology. 2002;**83**(Pt 4):829-833

[137] Jansma AL, Martinez-Yamout MA, Liao R, Sun P, Dyson HJ, Wright PE. The high-risk HPV16 E7 oncoprotein mediates interaction between the transcriptional coactivator CBP and the retinoblastoma protein pRb. Journal of Molecular Biology. 2014;**426**(24): 4030-4048

[138] Hangauer MJ, Vaughn IW, McManus MT. Pervasive transcription of the human genome produces thousands of previously unidentified long intergenic noncoding RNAs. PLoS Genetics. 2013;**9**(6):e1003569

[139] Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, et al. Landscape of transcription in human cells. Nature. 2012;**489**(7414): 101-108

[140] Guennewig B, Cooper AA. The central role of noncoding RNA in the brain. International Review of Neurobiology. 2014;**116**:153-194

[141] Pereira PM, Marques JP, Soares AR, Carreto L, Santos MA. MicroRNA expression variability in human cervical tissues. PLoS One. 2010; **5**(7):e11780

[142] Chen Y, Wang CX, Sun XX, Wang C, Liu TF, Wang DJ. Long non-coding RNA CCHE1 overexpression predicts a poor prognosis for cervical cancer. European Review for Medical and Pharmacological Sciences. 2017; **21**(3):479-483

[143] Yang M, Zhai X, Xia B, Wang Y, Lou G. Long noncoding RNA CCHE1 promotes cervical cancer cell proliferation via upregulating PCNA.

Tumour Biology: Journal of the International Society for Oncodevelopmental Biology and Medicine. 2015;**36**(10):7615-7622

[144] Barr JA, Hayes KE, Brownmiller T, Harold AD, Jagannathan R, Lockman PR, et al. Long non-coding RNA FAM83H-AS1 is regulated by human papillomavirus 16 E6 independently of p53 in cervical cancer cells. Scientific Reports. 2019;**9**(1):3662

[145] Lui WO, Pourmand N, Patterson BK, Fire A. Patterns of known and novel small RNAs in human cervical cancer. Cancer Research. 2007;**67**(13): 6031-6043

[146] Cao S, Liu W, Li F, Zhao W, Qin C. Decreased expression of lncRNA GAS5 predicts a poor prognosis in cervical cancer. International Journal of Clinical and Experimental Pathology. 2014; **7**(10):6776-6783

[147] Hu X, Schwarz JK, Lewis JS Jr, Huettner PC, Rader JS, Deasy JO, et al. A microRNA expression signature for cervical cancer prognosis. Cancer Research. 2010;**70**(4):1441-1448

[148] Kim SJ, Park SE, Lee C, Lee SY, Jo JH, Kim JM, et al. Alterations in promoter usage and expression levels of insulin-like growth factor-II and H19 genes in cervical carcinoma exhibiting biallelic expression of IGF-II. Biochimica et Biophysica Acta. 2002; **1586**(3):307-315

[149] Sharma S, Mandal P, Sadhukhan T, Roy Chowdhury R, Ranjan Mondal N, Chakravarty B, et al. Bridging links between long noncoding RNA HOTAIR and HPV oncoprotein E7 in cervical cancer pathogenesis. Scientific Reports. 2015;**5**:11724

[150] Sehnal B, Zikan M, Nipcova M, Dusek L, Cibula D, Slama J. The association among cervical, anal, and oral HPV infections in high-risk and low-risk women. European Journal of Obstetrics and Gynecology and Reproductive Biology. 2019;**4**:100061

[151] Jiang S, Wang HL, Yang J. Low expression of long non-coding RNA LET inhibits carcinogenesis of cervical cancer. International Journal of Clinical and Experimental Pathology. 2015;**8**(1): 806-811

[152] Jiang Y, Li Y, Fang S, Jiang B, Qin C, Xie P, et al. The role of MALAT1 correlates with HPV in cervical cancer. Oncology Letters. 2014;**7**(6):2135-2141

[153] Zhang J, Yao T, Wang Y, Yu J, Liu Y, Lin Z. Long noncoding RNA MEG3 is downregulated in cervical cancer and affects cell proliferation and apoptosis by regulating miR-21. Cancer Biology and Therapy. 2016;**17**(1): 104-113

[154] Qin R, Chen Z, Ding Y, Hao J, Hu J, Guo F. Long non-coding RNA MEG3 inhibits the proliferation of cervical carcinoma cells through the induction of cell cycle arrest and apoptosis. Neoplasma. 2013;**60**(5):486-492

[155] Zhou D, Wu F, Cui Y, Wei F, Meng Q, Lv Q. Long non-coding RNA-OIS1 inhibits HPV-positive, but not HPV-negative cervical squamous cell carcinoma by upregulating MTK-1. Oncology Letters. 2019;**17**(3):2923-2930

[156] Iden M, Fye S, Li K, Chowdhury T, Ramchandran R, Rader JS. The lncRNA PVT1 contributes to the cervical cancer phenotype and associates with poor patient prognosis. PLoS One. 2016; **11**(5):e0156274

[157] Liu Q, Guo X, Que S, Yang X, Fan H, Liu M, et al. LncRNA RSU1P2 contributes to tumorigenesis by acting as a ceRNA against let-7a in cervical cancer cells. Oncotarget. 2017;**8**(27): 43768-43781

[158] Kang HW, Wang F, Wei Q, Zhao YF, Liu M, Li X, et al. miR-20a promotes migration and invasion by regulating TNKS2 in human cervical cancer cells. FEBS Letters. 2012;**586**(6): 897-904

[159] Cao Y, Liu Y, Lu X, Wang Y, Qiao H, Liu M. Upregulation of long noncoding RNA SPRY4-IT1 correlates with tumor progression and poor prognosis in cervical cancer. FEBS Open Bio. 2016;**6**(9):954-960

[160] Chen X, Liu L, Zhu W. Up-regulation of long non-coding RNA CCAT2 correlates with tumor metastasis and poor prognosis in cervical squamous cell cancer patients. International Journal of Clinical and Experimental Pathology. 2015;**8**(10):13261-13266

[161] Wu L, Jin L, Zhang W, Zhang L. Roles of long non-coding RNA CCAT2 in cervical cancer cell growth and apoptosis. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2016;**22**:875-879

[162] Kawakami T, Zhang C, Taniguchi T, Kim CJ, Okada Y, Sugihara H, et al. Characterization of loss-of-inactive X in Klinefelter syndrome and female-derived cancer cells. Oncogene. 2004;**23**(36):6163-6169

[163] Sharma S, Munger K. Expression of the cervical carcinoma expressed PCNA regulatory (CCEPR) long noncoding RNA is driven by the human papillomavirus E6 protein and modulates cell proliferation independent of PCNA. Virology. 2018;**518**:8-13

[164] Liao LM, Sun XY, Liu AW, Wu JB, Cheng XL, Lin JX, et al. Low expression of long noncoding XLOC_010588 indicates a poor prognosis and promotes proliferation through upregulation of c-Myc in cervical cancer. Gynecologic Oncology. 2014;**133**(3):616-623

[165] Lee JW, Choi CH, Choi JJ, Park YA, Kim SJ, Hwang SY, et al. Altered microRNA expression in cervical carcinomas. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research. 2008; **14**(9):2535-2542

[166] Wang X, Tang S, Le SY, Lu R, Rader JS, Meyers C, et al. Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth. PLoS One. 2008;**3**(7):e2557

[167] Li Y, Wang F, Xu J, Ye F, Shen Y, Zhou J, et al. Progressive miRNA expression profiles in cervical carcinogenesis and identification of HPV-related target genes for miR-29. The Journal of pathology. 2011;**224**(4): 484-495

[168] Lajer CB, Garnaes E, Friis-Hansen L, Norrild B, Therkildsen MH, Glud M, et al. The role of miRNAs in human papilloma virus (HPV)-associated cancers: Bridging between HPV-related head and neck cancer and cervical cancer. British Journal of Cancer. 2012; **106**(9):1526-1534

[169] Liu S, Song L, Zeng S, Zhang L. MALAT1-miR-124-RBG2 axis is involved in growth and invasion of HR-HPV-positive cervical cancer cells. Tumour Biology: Journal of the International Society for Oncodevelopmental Biology and Medicine. 2016;**37**(1):633-640

[170] Martinez I, Gardiner AS, Board KF, Monzon FA, Edwards RP, Khan SA. Human papillomavirus type 16 reduces the expression of microRNA-218 in cervical carcinoma cells. Oncogene. 2008;**27**(18):2575-2582

[171] Xu Z, Zhou Y, Shi F, Cao Y, Dinh TLA, Wan J, et al. Investigation of differentially-expressed microRNAs and genes in cervical cancer using an

integrated bioinformatics analysis. Oncology Letters. 2017;**13**(4):2784-2790

[172] Gardiner AS, McBee WC, Edwards RP, Austin M, Lesnock JL, Bhargava R, et al. MicroRNA analysis in human papillomavirus (HPV)-associated cervical neoplasia and cancer. Infectious Agents and Cancer. 2010;**5**(1):A55

[173] Bodaghi S, Jia R, Zheng ZM. Human papillomavirus type 16 E2 and E6 are RNA-binding proteins and inhibit in vitro splicing of pre-mRNAs with suboptimal splice sites. Virology. 2009;**386**(1):32-43

[174] Yeung CL, Tsang TY, Yau PL, Kwok TT. Human papillomavirus type 16 E6 suppresses microRNA-23b expression in human cervical cancer cells through DNA methylation of the host gene C9orf3. Oncotarget. 2017;**8**(7):12158-12173

[175] Jung HM, Phillips BL, Chan EK. miR-375 activates p21 and suppresses telomerase activity by coordinately regulating HPV E6/E7, E6AP, CIP2A, and 14-3-3zeta. Molecular Cancer. 2014;**13**:80

[176] Morel A, Baguet A, Perrard J, Demeret C, Jacquin E, Guenat D, et al. 5azadC treatment upregulates miR-375 level and represses HPV16 E6 expression. Oncotarget. 2017;**8**(28):46163-46176

[177] Wang F, Li Y, Zhou J, Xu J, Peng C, Ye F, et al. miR-375 is down-regulated in squamous cervical cancer and inhibits cell migration and invasion via targeting transcription factor SP1. The American Journal of Pathology. 2011;**179**(5):2580-2588

[178] Liu S, Song L, Yao H, Zhang L, Xu D, Gao F, et al. MiR-375 is epigenetically downregulated by HPV-16 E6 mediated DNMT1 upregulation

and modulates EMT of cervical cancer cells by suppressing lncRNA MALAT1. PLoS One. 2016;**11**(9):e0163460

[179] Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, et al. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**(28):11667-11672

[180] Lu H, He Y, Lin L, Qi Z, Ma L, Li L, et al. Long non-coding RNA MALAT1 modulates radiosensitivity of HR-HPV+ cervical cancer via sponging miR-145. Tumour Biology: Journal of the International Society for Oncodevelopmental Biology and Medicine. 2016;**37**(2):1683-1691

[181] Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, et al. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell. 2007;**129**(7):1311-1323

[182] Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature. 2010;**464**(7291):1071-1076

[183] Zhang M, Song Y, Zhai F. ARFHPV E7 oncogene, lncRNA HOTAIR, miR-331-3p and its target, NRP2, form a negative feedback loop to regulate the apoptosis in the tumorigenesis in HPV positive cervical cancer. Journal of Cellular Biochemistry. 2018;**119**(6):4397-4407

[184] Fujii T, Shimada K, Asano A, Tatsumi Y, Yamaguchi N, Yamazaki M, et al. MicroRNA-331-3p suppresses cervical cancer cell proliferation and E6/E7 expression by targeting NRP2. International Journal of Molecular Sciences. 2016;**17**(8)

[185] Zambrano P, Segura-Pacheco B, Perez-Cardenas E, Cetina L, Revilla-Vazquez A, Taja-Chayeb L, et al. A phase I study of hydralazine to demethylate and reactivate the expression of tumor suppressor genes. BMC Cancer. 2005;**5**:44

[186] de la Cruz-Hernandez E, Perez-Cardenas E, Contreras-Paredes A, Cantu D, Mohar A, Lizano M, et al. The effects of DNA methylation and histone deacetylase inhibitors on human papillomavirus early gene expression in cervical cancer, an in vitro and clinical study. Virology Journal. 2007;**4**:18

[187] You JS, Kang JK, Lee EK, Lee JC, Lee SH, Jeon YJ, et al. Histone deacetylase inhibitor apicidin downregulates DNA methyltransferase 1 expression and induces repressive histone modifications via recruitment of corepressor complex to promoter region in human cervix cancer cells. Oncogene. 2008;**27**(10):1376-1386

[188] Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, et al. Quercetin, inflammation and immunity. Nutrients. 2016;**8**(3):167

[189] Kedhari Sundaram M, Hussain A, Haque S, Raina R, Afroze N. Quercetin modifies 5'CpG promoter methylation and reactivates various tumor suppressor genes by modulating epigenetic marks in human cervical cancer cells. Journal of Cellular Biochemistry. 2019

[190] Lewis A, Kang R, Levine A, Maghami E. The new face of head and neck cancer: The HPV epidemic. Oncology. 2015;**29**(9):616-626

[191] Kim SM. Human papilloma virus in oral cancer. Journal of the Korean Association of Oral and Maxillofacial Surgeons. 2016;**42**(6):327-336

[192] Hernandez BY, McDuffie K, Zhu X, Wilkens LR, Killeen J, Kessel B, et al. Anal human papillomavirus infection in women and its relationship with cervical infection. Cancer Epidemiology, Biomarkers and Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology. 2005;**14**(11 Pt 1):2550-2556