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Regulation of cellular miRNA expression by human papillomaviruses

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Abstract

High-risk HPV infection leads to aberrant expression of cellular oncogenic and tumor suppressive miRNAs. A large number of these miRNA genes are downstream targets of the transcription factors c-Myc, p53, and E2F and their expression can therefore be modulated by oncogenic HPV E6 and E7. Cervical cancer represents an unique tumor model for understanding how viral E6 and E7 oncoproteins deregulate the expression of the miR-15/16 cluster, miR-17-92 family, miR-21, miR-23b, miR-34a, and miR-106b/93/25 cluster via the E6–p53 and E7–pRb pathways. Moreover, miRNAs may influence the expression of papillomavirus genes in a differentiation-dependent manner by targeting viral RNA transcripts. Cellular miRNAs affecting HPV DNA replication are of great interest and will be a future focus. We are entering an era focusing on miRNA and noncoding RNA, and the study of HPV and host miRNA interactions will continue to shed more light on our understanding of the HPV life cycle and the mechanistic underpinnings of HPV-induced oncogenesis.

Keywords

human papillomaviruses; microRNAs; oncogenes; tumor suppressor genes; cervical cancer; gene expression

Introduction

Human papillomaviruses (HPVs) are a group of small DNA tumor viruses ~55 nm in diameter. HPVs contain a small, double-stranded circular genome of ~8 kb and encode six viral early proteins (E6, E7, E1, E2, E4, and E5) that have regulatory functions and two viral late proteins (L2 and L1) for viral capsid formation [1]. Infection through sexual intercourse is initiated when a viral particle gains entry into a basal epithelial cell of the cervix. Viral gene expression and multiplication occur exclusively in the nuclei of the infected cells and are tightly linked to the state of differentiation of the cells. Viral early genes are expressed in the undifferentiated basal and parabasal layers, and expression of viral late genes and viral DNA replication occur in the upper spinous and more differentiated granular or cornified layers of the infected cervical epithelium (Fig. 1). Some HPVs, such as HPV16 and HPV18, are associated with oncogenesis and are therefore considered “high risk (HR)”. Viral E6 and

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E7 of HR HPVs are viral oncoproteins and respectively inactivate p53 and pRB, two major cellular tumor suppressors, thereby contributing to cervical carcinogenesis [2-4].

MicroRNAs (miRNAs) are noncoding regulatory RNAs 18-25 nucleotides in size that are derived from RNA polymerase II (pol II) transcripts of coding or noncoding genes. Many miRNAs are tissue- or differentiation-specific, and their temporal or short-lived expression modulates gene expression at the posttranscriptional level by base-pairing with complementary nucleotide sequences (seed matching) of target mRNAs [5;6]. Depending on the degree of sequence complimentarity, the binding of miRNA(s) to a target mRNA inhibits protein translation, degrades the target mRNA, or both. As of September 2010, the miRBase database (http://www.mirbase.org/) had collected 15,172 entries representing hairpin precursor pre-miRNAs expressing 17,341 mature miRNAs in 143 species. Human genome contains ~416 miRNA genes encoding 1048 distinct mature miRNAs from every chromosome except Y (Fig. 2). Approximately, ~113 miRNA genes encode a cluster of miRNAs and produce ~390 miRNA sequences. Bioinformatics prediction shows that each miRNA targets ~200 RNA transcripts directly or indirectly, and up to one-third of the total number of human mRNAs are targets of more than one miRNA [7;8]. Thus, the actions of miRNAs exert profound effects on gene expression at the posttranscriptional level in almost every biological process. However, miRNA expression itself, similar to any other transcription mediated by pol II, is regulated both at the transcriptional and posttranscriptional levels. Many cellular transcription factors, including c-Myc, p53, and E2F, have been described to regulate miRNA transcription. Other factors involved in miRNA maturation and processing after transcription are Drosha (an RNase-III endonuclease that produces pre-miRNA from pri-miRNA), DGCR8 (DiGeorge syndrome critical region gene 8, a double-stranded RNA-binding protein needed for Drosha activity), exportin 5 (for pre-miRNA export), Dicer (an RNase-III enzyme that produces mature miRNA from pre-miRNA), TRBP (a Dicer partner), and Ago2 (a major component of RISC) [9;10]. Because oncogenic HPV E6 induces degradation of p53 and E7 mediates degradation of pRB to release E2F from the pRB-E2F complex, it is conceivable that oncogenic HPV infection causes aberrant expression of cellular miRNAs.

Modulation of cellular miRNA expression by c-Myc, p53, and E2F, which are regulated by oncogenic HPV E6 and E7

High-risk E6 interacts with c-Myc to enhance c-Myc binding to the hTERT (human telomerase reverse transcriptase) promoter and induces hTERT mRNA transcription [11-13]. Viral E7 induces the expression of c-Myc by binding pRB protein, thereby releasing E2F to activate c-Myc [14-16]. High-risk E7 also interacts directly with c-Myc [17]. Myc is a basic helix–loop–helix leucine zipper protein that dimerizes with Max to bind the DNA sequence CACGTG, known as an E box, and thereby activates gene transcription [18;19]. c-Myc is a potent transcriptional regulator of miRNA expression [20;21]. c-Myc induces the expression of the miR-17-92 family on chromosome 13 and of E2F1, but miR-17-5p and miR-20a in the family target the E2F1 3′ UTR to prevent its efficient translation [20;21]. However, c-Myc overexpression widely represses miRNA expression. Chromatin immunoprecipitation reveals that much of this repression is likely to be a direct result of c-Myc binding to miRNA promoters, including those of let-7a-1/f-1/d, miR-15a/16-1, miR-22, miR-26a-2, miR-26b, miR-29a/b-1, miR-29b-2/c, miR-30e/30c-1, miR-34a, and miR-146a [21].

Oncogenic E6 targets p53 for degradation via the E6AP ubiquitin proteolytic pathway [22;23]. The tumor suppressor p53 is a transcription factor that binds to a promoter=s p53 binding site (a palindrome DNA sequence of RRRCWWGYYY [R=A,G;W=A,T;Y=C,T]) [24]. It regulates many cellular miRNAs by increasing the expression of miR-23a, miR-26a, and miR-34a [25] via direct transactivation of these miRNA genes. Other studies show that

p53 decreases expression of miRNA clusters, including miR-106b/miR-93/miR-25, miR-17-5p/18a/19a/20a/19b-1/92-1, and miR-106a/18b/20b/19b-2/92-2 [26]. Interestingly, p53 decreases expression of the miRNA clusters by an indirect mechanism through repression of E2F1 [26]. It has been reported that p53 transactivates expression of the BTG3 (B-cell translocation gene 3) gene, which directly binds E2F1 and inhibits its activity [27]. p53-induced miR-34a also targets the E2F1 3′ UTR and inhibits its expression [28]. Moreover, p53 interacts with the Drosha/p68 complex to facilitate Drosha-mediated pri-miRNA processing, consequently promoting expression of miR-15a/16-1, miR-103/107, miR-143/145, miR-203, and miR-206 at the posttranscriptional level [25].

As noted above, E7-mediated degradation of tumor suppressor protein pRB frees E2F from the pRB-E2F complex. The promoter regions of many miRNA genes contain an E2F binding site (TTTSSCGC, S= C or G) [29], and binding of E2F to an E2F binding site in the promoter region transactivates the expression of miRNA genes including miR-17-92, let-7a-d, let-7i, miR-15b/16-2, and miR-106b-25 [20;30;31]. In addition, E2F1 directly activates the transcription of coding genes by binding to their promoter regions, and thus promotes miRNA expression from these transcripts. For example, E2F1 promotes MCM7 expression from chromosome 7q22 and production of miR-106b/93/25 from its intron 13 [32].

miRNA signatures in cervical cancer

Cervical cancer ranks as the most common cancer in women in the developing world, with an estimated global incidence of 493,243 cases and approximately 273,505 deaths per year (www.who.int/hpvcentre). Cervical cancer is the fourth most common type of cancers in women in the U.S., with an estimated 12,200 new cases in 2010, and is the eighth leading cause of cancer mortality in women, accounting for an estimated 4,210 deaths in 2010 [33]. Sexual infection with oncogenic HPVs is widely recognized as a leading cause of cervical, penile, and anal cancers. Among over 120 genotypes isolated from humans [34], oncogenic HPVs, such as HPV16, HPV18, and HPV31, have been detected in up to 99.7% of cervical squamous cell carcinomas and 94%-100% of cervical adeno- and adenosquamous carcinomas [35;36].

Genome-wide profiling of miRNA signatures have indicated that aberrant (increased or decreased) miRNA expression is common in most human tumors [10;37]. Besides regulating cell cycle progression, proliferation/differentiation, apoptosis, and senescence, miRNAs have been linked to cancer etiology, progression, metastasis, and prognosis and could function as oncogenes (e.g., miR-17-92, miR-21, miR155, miR-372/373) or tumor suppressors (e.g., let-7, miR-15a/16-1, miR-34a, miR-143/145) by modulating oncogenic or tumor suppressive pathways, including the Ras, Myc, p53, and pRB pathways. Some miRNAs, such as the miR-221/222 and miR-17-92 clusters, may be oncogenic in one cell or tissue type but tumor suppressive in another, depending on the tissue context and target genes [37]. Moreover, the tumor suppressive miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-149) and tumor suppressive miR-205 have been identified as controlling cancer metastasis [38-40].

In the course of understanding how HPV16 develops resistance to E7-specific siRNA, in 2004 we isolated and cloned E7-specific siRNAs derived from shRNA precursors [41] and 174 cellular miRNAs from HPV16$^+$ CaSki cells [42]. In combination with Northern blot and miRNA array analyses of 11 cervical cancer cell lines and cervical cancer tissues, as well as of HPV16-induced pre-neoplastic lesions in raft tissues derived from primary human vaginal keratinocytes, we concluded that a substantial number of cellular miRNAs exhibited altered expression due to HPV infection (Table 1) [42]. A recent study by Li et al. [43] independently analyzed six paired normal and cervical cancer tissues by using the same

miRNA array platform and showed a very similar result. Increased expression of 12 miRNAs (miR-15b, miR-16, miR-17-5p, miR-20a, miR-20b, miR-21, miR-93, miR-106a, miR-155, miR-182, miR-185, and miR-224) and decreased expression of 9 miRNAs (miR-29a, miR-34a, miR-126, miR-127, miR-145, miR-218, miR-424, miR-450, and miR-455) were found in cervical cancer tissues in both laboratories (Table 1) [42-45]. Among the altered miRNAs reported by both laboratories, miR-126, miR-143/145, miR-155, miR-424/450 were also confirmed by deep sequencing (Table 1)[46]; increased expression of miR-20a, miR-20b, miR-93, and miR-224 and decreased expression of miR-127, miR-143/145, and miR-218 were confirmed in cervical cancer tissues when compared with adjacent normal cervical tissues (2 cm away from the cancerous tissues) using a different miRNA array platform [47]. Other studies with customized miRNA arrays [48-50] or different assay platforms [51] revealed more variable results. However, one study [51] found increased expression of miR-21 in cervical cancer, and two others [48;51] found decreased expression of both miR-143 and miR-145. Together, the data indicate that cervical cancer expresses no or very little of the miR-143/145 cluster. However, reduced miR-143/145 expression is common in other tumor types unrelated to HPV infection [52;53]. The miR-143/145 cluster is a class II miRNA whose expression is unrelated to any E2F factor and is greatly increased during G1 and maintained or further increased in proliferating cells [30]. A recent study indicates that downregulation of the miR-143/145 cluster requires the Ras-responsive element-binding protein (RREB1) to repress its promoter, but K-Ras and RREB1 are themselves targets of miR-143/145, arguing for a feed-forward mechanism that potentiates Ras signaling [54].

Using a PCR-based miRNA assay to analyze 102 cervical cancer samples, Hu et al. identified miR-200a and miR-9 as two promising miRNAs that could be used to predict cervical cancer survival [50]. However, in other studies neither miR-9 nor miR-200a appeared to be expressed at significantly different levels in cervical cancer tissues than in age-matched normal cervical tissues [42;43;46]. In one report, the altered miR-200a expression showed no correlation with cervical cancer invasion and metastasis [47]. Thus, further independent analyses are needed to validate the reported observation [50].

HPV E6 regulates the expression of miR-23b, miR-34a, and miR-218

The major function of oncogenic HPV E6 is to target p53 for degradation. Structural and functional analyses of HPV16 E6 indicate that an E6 F47R mutant is defective for polyubiquitination and degradation of p53 [55;56]. As a transcription factor, p53 plays an important role in the transcription of numerous coding and noncoding genes [25;57;58]. Conceivably, oncogenic HPV E6 is capable of regulating the expression of many cellular miRNAs via p53. We initially observed this in our laboratory when we were investigating how high-risk HPV infection leads to reduction of miR-34a expression [44]. Because the miR-34a gene is a direct transcriptional target of p53, and its expression can be transactivated by the binding of p53 to a consensus p53 binding site in the miR-34a promoter region [59-61], we and others found that E6-mediated degradation of p53, and not E7-mediated reduction of pRB, leads to the reduction of miR-34a that is mediated by high-risk HPV infection in raft cultures, cervical intraepithelial neoplasia (CIN), and cervical cancer tissues. Conversely, knockdown of viral E6 expression in $HPV16^+$ and $HPV18^+$ cervical cancer cell lines by siRNAs increases the expression of p53 and also of miR-34a [44;45]. A direct link between p53 and miR-34a expression was convincingly achieved in C33A cells, an HPV-negative cervical cancer cell line expressing a mutant p53 and producing no miR-34a. Ectopic expression of wild-type p53 in C33A cells induces production of miR-34a [44]. Because miR-34a affects the expression of cell cycle regulators, including cyclin E2, cyclin D1, CDK4, CDK6, E2F1, E2F3, E2F5, Bcl-2, SIRT1, and p18Ink4c [28;59-65], viral E6–mediated reduction of p53 and miR-34a quickly relieves the

multi-step controls on cell cycle progression, senescence, and apoptosis, resulting in cell proliferation and transformation (Fig. 3). However, recent studies indicate that p53-independent upregulation of miR-34a can be triggered in cells undergoing terminal differentiation [44] or senescence [66]. In contrast, cancer cells may inactivate miR-34a expression by aberrant CpG methylation [67] independent of HPV E6.

Viral E6 also regulates expression of miR-218 and miR-23b. The reduction of miR-218 by oncogenic HPV E6 leads to increased LAMB3 (Laminin subunit beta-3) production and appears to be unrelated to p53, because miR-218 is highly expressed in the HPV-negative C33A cells containing a mutant p53 gene and producing no miR-34a [68]. Although how E6 reduces miR-218 expression remains unknown, HPV16 E6 reduction of miR-23b has been linked to p53. Au Yeung and colleagues found that downregulation of miR-23b expression by HPV16 E6 results in an increased expression of urokinase-type plasminogen activator (uPA) [69;70] and is related to E6-mediated p53 reduction [71]. Similar to miR-34a, the gene encoding miR-23b on chromosome 9 contains a promoter region with a p53 binding site [71], and in the presence of p53, expresses miR-23b as a cluster of miR-23b/27b/24-1. The data provide miR-23b as another example that p53 mediates the HPV16 E6 downregulation of cellular miRNAs (Fig. 3).

HPV E7 regulates the expression of miR-15a/miR-16-1 and miR-203

Tumor suppressive miR-15a and miR-16-1 control cell proliferation, survival, and invasion [72-74] and are expressed as an miRNA cluster from an intron region of the DLEU2 (deleted in lymphocytic leukemia 2) transcript (Fig. 4). The gene encoding the miR-15a/16-1 cluster is a noncoding DLEU2 gene and is positioned in the antisense orientation in the 13q14.3 locus, which is frequently deleted in chronic lymphocytic leukemia (CLL) [75;76]. In mouse, a deletion of DLEU2/miR-15a/16-1 from the 14qC3 region, a conserved region equivalent to human 13q14, also accelerates the proliferation of mouse B cells by modulating the expression of genes controlling cell-cycle progression, further defining the role of 13q14 deletions in the pathogenesis of CLL [77]. Although both miR-15a and miR-16-1 are abundantly expressed in normal tissues, studies of cancer-related miRNA signatures indicate that the miR-15a/16-1 cluster is downregulated in multiple types of human cancer [78]. However, our initial study using miRNA array analyses showed higher levels of miR-15a and miR-16-1 expression in cervical cancer tissues than in normal cervical tissue [42]. Despite increased expression in cervical cancer, miR-15a and miR-16-1 appear not to function efficiently in controlling the growth of cervical cancer cells.

To investigate whether increased expression of the miR-15a/16-1 cluster in cervical cancer tissues is related to high-risk HPV infection, we used Northern blotting to compare the expression levels of miR-16-1 in raft tissues derived from human foreskin keratinocytes with and without HPV18 infection and observed a more than two-fold increase of miR-16-1 expression in the rafts with HPV18 infection over the expression in control rafts without HPV18 infection (Fig. 5A). When rafts infected with a retrovirus expressing HPV18 E6, E7, or E6E7 were examined, the production of miR-16-1 was increased only in the rafts expressing viral E7, not viral E6 (Fig. 5B), indicating that viral E7 is responsible for the increased expression of miR-16-1. The reverse was true in cervical cancer cell lines with decreased expression of viral E7: by knocking down viral E7 expression with RNAi in $HPV16^+$ CaSki cells or $HPV18^+$ HeLa cells, we demonstrated decreased expression of miR-16-1 in the cells treated with an E7-specific siRNA compared to the control cells treated with a non-specific siRNA (Fig. 5C). Together, these data lead to the conclusion that viral E7 regulates miR-15/16 cluster expression in cells with HPV16 or HPV18 infection. The expression of miR-15a/16-1 is controlled by binding of c-Myc, c-Myb, or PPAR (peroxisome proliferator-activated receptor δ) to the DLEU2 promoter region to positively

(c-Myb and PPAR) or negatively (c-Myc) regulate DLEU2 transcription [21;79;80]. E2F1 transactivates c-Myb expression [81;82] but represses c-Myc expression [83]. Given these relationships, it is understandable that the increased expression of miR-15a and miR-16-1 in cervical cancer tissues could be attributed to viral E7–mediated degradation of the tumor suppressor pRB. Viral E7–mediated degradation of pRB [4] frees E2F from the pRB-E2F complex to interact with the promoter regions of c-Myb and c-Myc, consequently regulating DLEU2 transcription and promoting miR-15a/16-1 cluster expression. In addition to transcriptional regulation of DLEU2 gene expression, however, posttranscriptional regulation may also play an important role in the expression of miR-15a and miR-16-1. Because miR-15a and miR-16-1 are derived from the intron 3 region of the DLEU2-001 transcript (Fig. 4), it remains to be understood how RNA splicing contributes to the Drosha-dependent production of miR-15a and miR-16-1 from the DLEU2-001 transcripts during high-risk HPV infection of cervical tissues.

High-risk HPV E7 in human keratinocytes downregulates the expression of miR-203 [84] which controls the shift of keratinocytes in differentiating epithelia from a proliferative state to a nonproliferative state by repressing stemness [85;86]. Although primarily expressed in superbasal layers of stratified epithelia, miR-203 expression can be induced in vitro in primary keratinocytes in parallel with differentiation. In normal human foreskin keratinocytes with stable expression of HPV31 episomes or HPV31 E6 or E7, differentiation-dependent miR-203 expression is severely blocked only by viral E7, with a corresponding increase in expression of ΔNp63, a member of the p53 family that is highly expressed in proliferative undifferentiated basal keratinocytes, but poorly expressed in differentiated nonproliferative cells [86;87]. Viral E7 perhaps blocks the MAPK/PKC pathway–dependent activation of miR-203 expression [84]. Other studies have shown that the primary role of miR-203 is to inhibit the proliferative capacity of epithelial cells upon differentiation by targeting the 3′ UTR regions of ΔNp63 [86;87].

Cellular miRNAs regulate HPV gene expression and replication

Oncogenic HPVs regulate the expression of many cellular miRNAs; conversely, it is now emerging that cellular miRNAs modulate the expression of HPV genes. As every viral gene transcript derived from the HPV genome is in a bicistronic or polycistronic form, with a long 5′ UTR or 3′ UTR, we have hypothesized that HPVs express their genes under the control of multiple cellular miRNAs [1]. In fact, ectopic expression of any given HPV gene has been a challenge; cervical tissues with high-risk HPV infection express very little viral protein, which in general practice is scarcely detectable. Codon optimization to remove “the suppressive nucleotides” (presumably the nucleotides in miRNA seed matches) of a given viral transcript was introduced to enhance the expression of viral genes and has been a common practice in papillomavirology. We recently analyzed the genome-wide distribution of miRNA binding sites of ~450 hsa-miRNAs (homo sapiens miRNAs) in the HPV6, HPV11, HPV16, and HPV18 genomes and identified several dozen potential miRNA binding sites in each HPV gene transcript. Our preliminary results indicate that expression of both HPV early and late genes is subject to miRNA-mediated regulation at the post-transcriptional level in cervical cancer cell lines and in reporter assays (unpublished observations).

Interestingly, Melar-New and Laimins [84] recently found that a differentiation-dependent miR-203 increase in CIN612 cells containing HPV31 episomes promotes viral genome amplification in the short term, but a high level of miR-203 expression interferes with viral genome amplification in the long term [84]. Although the mechanism by which miR-203 is involved in viral DNA replication remains to be investigated, this study provides the first evidence that cellular miRNAs may also play an important role in regulation of viral gene

expression and DNA replication. In a separate study, HPV16 DNA replication was suppressed by ectopic miR-125b, presumably through the sequence homology of HPV16 L2 and miR-125b. Conversely, reduced miR-125b expression was found in cervical pre-cancerous lesions during early HPV infection and may also be associated with viral DNA replication [88]. Together, the current data are not yet sufficient to conclude that HPV gene expression is under massive regulation by cellular miRNAs, but this presents a valuable direction for understanding HPV biology and pathogenesis.

Conclusions and Remarks

Cervical cancer, like many other cancer types [10;37], displays notably increased or decreased expression of a large number of cellular oncogenic or tumor suppressive miRNAs (Table 1). Because tumor suppressive miRNAs with decreased expression are more numerous in cervical cancer tissues than oncogenic miRNAs with increased expression (Table 2), the altered miRNA expression appears unlikely to be attributable to Drosha overexpression from the chromosome 5p gain in cervical cancer [89;90]. It is clear that oncogenic HPVs, despite producing no viral miRNAs [42;91], are responsible for the aberrant expression of oncogenic or tumor suppressive miRNAs. Although these observations shed more light on the mechanistic underpinnings of HPV-induced oncogenesis (Fig. 6), whether the altered miRNA expression would sensitize the HPV genome to integration or infected cells to unrestricted proliferation remains to be discovered.

To date, mounting evidence indicates that HPV regulation of cellular miRNA expression most likely occurs through viral E6 and E7, although other viral protein(s) might be involved. In this regard, E6 degradation of p53 contributes to reduced expression of miR-34a [42;45] and miR-23b [71] at the transcriptional level. However, the mechanism by which E6 reduces miR-218 expression and how E7 regulates the expression of miR-203 and the miR-15/16 cluster remain elusive. High-risk E6 and E7 interact separately with several dozens or even hundreds of cellular factors [92-94], and these interactions could lead to increased or decreased expression of cellular miRNAs. Some of the altered miRNA expression could be a result from both E6 and E7. The matter is to discover what these miRNAs are and how E6 and E7 regulate them.

Increased miR-21 expression in cervical cancer was noticed in several studies [42;43;51] and may be attributable to both E6 and E7. Expression of miR-21 in human and mouse cells depends on STAT3 and p65 NF-κB binding to the miR-21 promoter region [95;96]. Consistent with this, cervical cancer tissues display an increased amount of STAT3 [97-99] and high-risk HPV E6 and E7 increase p65 NF-κB expression [100]. Moreover, miR-21 targets the PTEN tumor suppressor, also leading to increased NF-κB activity [96] and inhibits negative regulators of the Ras/MeK/ERK pathway to block apoptosis [101], feeding forward to viral E6 and E7 functions. Together, these reports help to solve a long-standing puzzle of how E7 could operate with activated Ras to fully transform primary rat cells [102;103].

Considering that each miRNA subtly affects hundreds of different gene transcripts for protein expression, HPV transcripts could be targeted in turn by these altered miRNAs in a miRNA-directed negative feedback loop. Notably, inefficient translation of HPV16 transcripts has been thought to be due to rare codon usage in the viral open reading frames; therefore, codon optimization, which may disrupt the miRNA binding sites, has been introduced to improve the production of various HPV16 proteins [1]. Thus, future studies should elucidate the effects of such miRNAs on HPV protein expression (Fig. 6). The altered miRNAs may also deregulate downstream targets of viral E6 or E7. For example, high-risk E6 and E7 increase MCM7 expression through E2F1-dependent and -independent

pathways [104;105]. Production of the MCM7 transcripts that produce the miR-106b/93/25 cluster decreases along with the increase of p53 expression, because p53 induces transcriptional repression of E2F1 [26]. Because viral E6 degrades p53 and E7 interacts with pRB to release E2F1 from the pRB-E2F complex, increased expression of the miR-106b/93/25 cluster is expected in cervical cancer tissues with high-risk HPV infection. However, both miR-106b and miR-93 target and negatively regulate the expression of p21 and E2F1 [32]. The finding of miR-93 upregulation in cervical cancer tissues in three separate studies [42;43;47] indicates that the circuit favors the transactivation of MCM7 expression and miR-106b/93/25 cluster production by E2F1 due to viral E6 and E7.

Although the viral oncoproteins E6 and E7 play important roles in regulating cellular miRNA expression, their function can be reduced due to epigenetic modification of miRNA genes. Because miRNA genes are pol II genes [106], their transcription is driven by a pol II promoter, similar to all other eukaryotic coding genes. Therefore, aberrant CpG methylation and histone modification, which affect expression of coding genes, will also influence the expression of miRNA genes [107-109] including those under regulation by viral E6 and/or E7. DNA methylation–mediated silencing of miR-34a expression has been reported in multiple tumor types [67]. CpG island hypermethylation is a hallmark of cervical cancer [110-112]. Consistent with this, a recent report indicates that miR-124 reduction in cervical cancer correlates with a high frequency of DNA methylation in the miR-124 locus [113].

There is no doubt that biomedicine is entering an era of miRNAs and non-coding RNAs. The widespread research in laboratories and clinical settings today relies heavily on microarray screening and deep sequencing of biological samples and qRT-PCR. As different miRNA array platforms are now commercially available, with variable qualities and sensitivities [114;115], special care should be taken when making a conclusion based on array data from a single platform. Dreher and colleagues have compared three different miRNA array platforms (Affymetrix, Invitrogen, and Exiqon) in profiling hsa-miRNA expression from HaCaT cells transfected with the full-length HPV-11 genome and found inconsistent results among the three platforms. They also show that TaqMan qRT-PCR validation is of limited use for less abundant miRNAs [116], which argues that qRT-PCR is not a “gold-standard” for miRNA validation [114] and cannot be used to replace the Northern blotting method [117]. Despite these technical issues, the study of miRNAs shows great promise and will be a prospective focus in coming years to further understand HPV life cycle and pathogenesis.

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Abbreviations

HPV	human papillomavirus
miRNA	microRNA
RISC	RNA-induced silencing complex
hTERT	human telomerase reverse transcriptase
BTG3	B-cell translocation gene 3
CIN	cervical intraepithelial neoplasia
uPA	urokinase-type plasminogen activator
DLEU2	deleted in lymphocytic leukemia 2
CLL	chronic lymphocytic leukemia
HFK	human foreskin keratinocytes
RREB1	Ras-responsive element-binding protein 1
PPAR	peroxisome proliferator-activated receptor

Research Highlights

Oncogenic HPV infection leads to development of cervical cancer and deregulates the expression of oncogenic and tumor suppresive miRNAs via E6-p53 and E7-pRb pathways. Cellular miRNAs may also influence the expression of papillomavirus genes in a differentiation-dependent manner by targeting viral RNA transcripts. Cervical cancer provides a unique cancer model for understanding the interplays between HPV oncogenes and host miRNAs and their roles in cervical carcinogenesis.

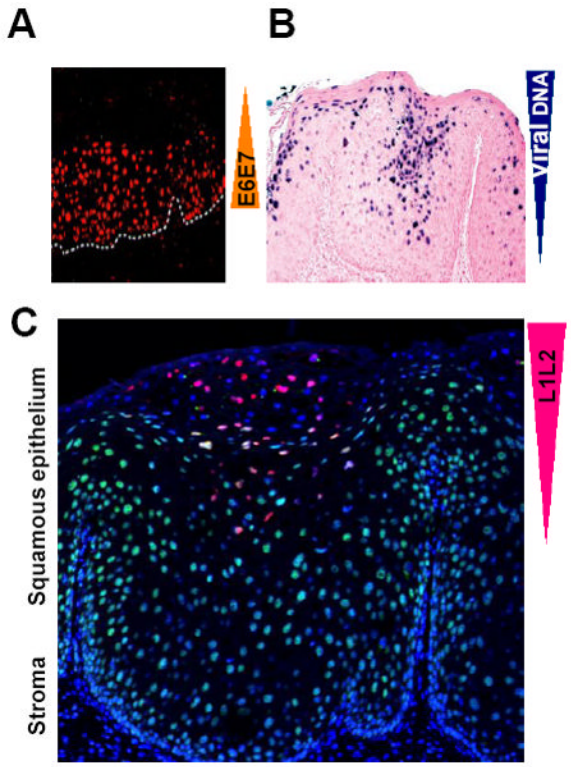

Fig. 1.

Keratinocyte differentiation–dependent HPV16 life cycle. In general, HPV16 infects the cervical basal cells through microtrauma during sexual intercourse and initiates viral E6 and E7 expression in the infected cells (orange color in A for MCM7 as a surrogate for viral E6 and E7, modified with permission from John Doorbar [104]). Viral DNA replication takes place in the spinous and granular keratinocytes under intermediate or high differentiation (navy blue color in B for viral DNA, modified with permission from Ming Guo[119]). However, viral L1 and L2 (red color for L1 in C) become detectable only in the granular and cornified keratinocytes under terminal differentiation (Jia R and Zheng ZM, unpublished observation) [120].

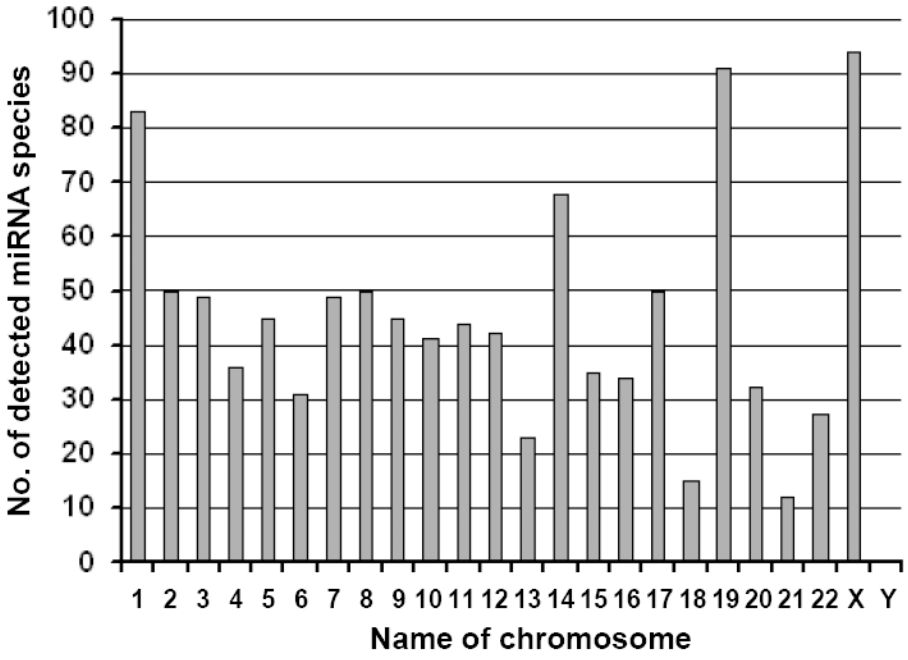

Fig. 2.
miRNA expression and human chromosomes. Data were obtained from the miRbase sequence database, release 16, September 2010 (http://www.mirbase.org/).

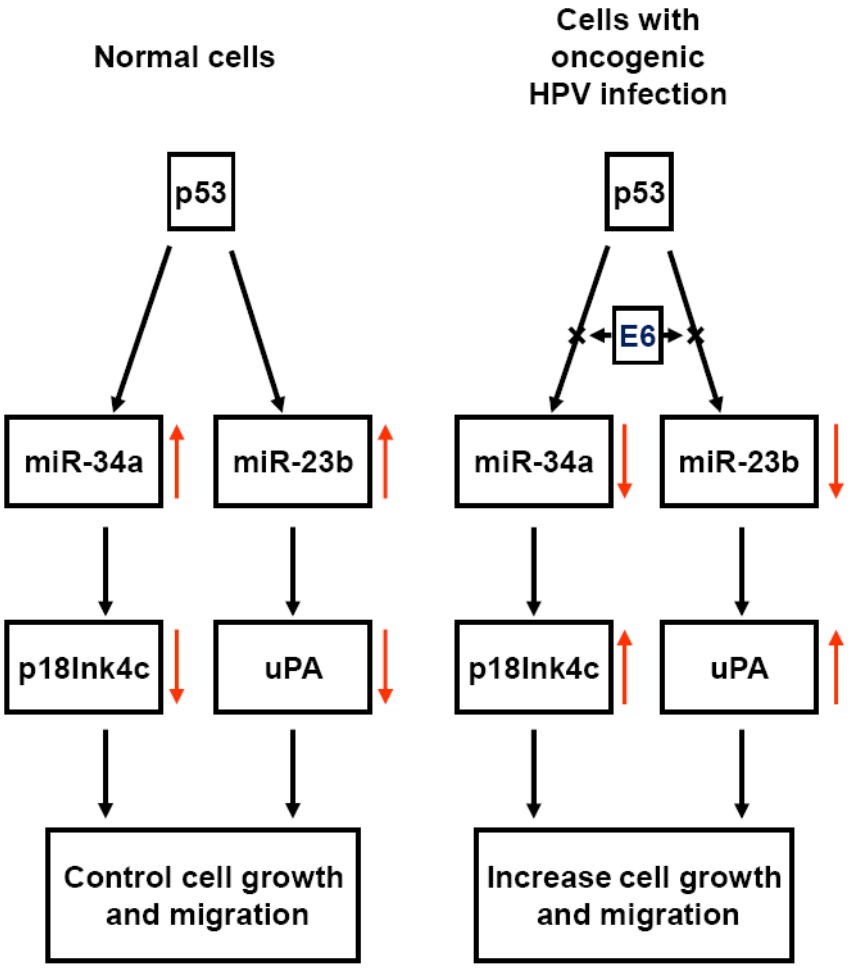

Fig. 3.
Oncogenic HPV regulates p53 expression and its downstream targets miR-34a and miR-23b. Arrows in color indicate expression level (increase or decrease).

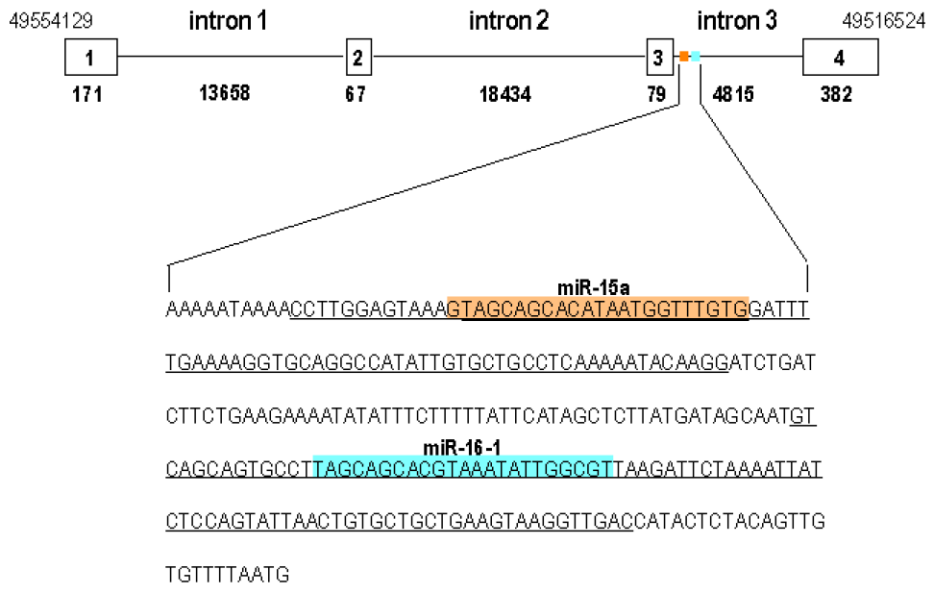

Fig. 4.

Production of the miR-15a/16-1 cluster from a DLEU-2-001 transcript containing 4 exons (boxes) and 3 introns (lines). Numbers below exons and introns indicate the size in nucleotides.

The underlines are miRNA stem-loop regions, with the miR-15a and miR-16-1 sequences in color.

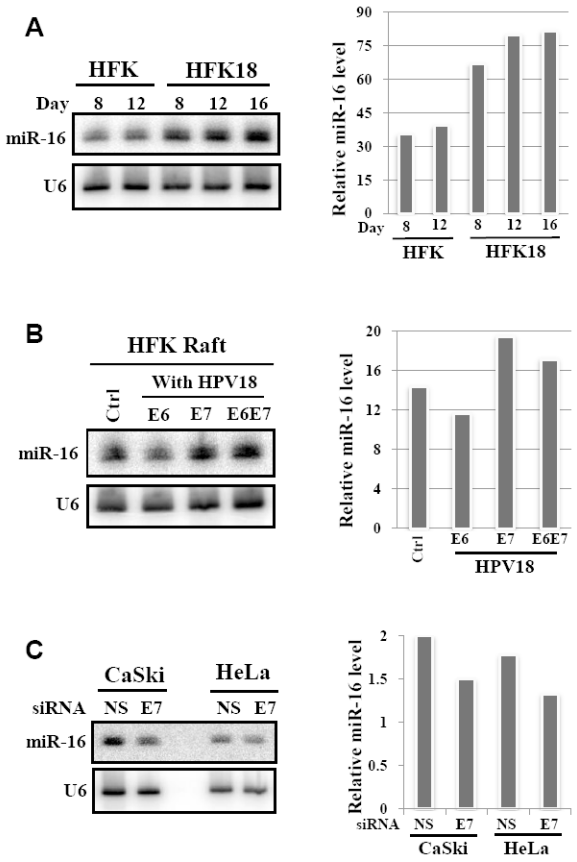

Fig. 5.

Oncogenic HPV infection increases miR-16 expression through viral E7. Northern blotting was used to detect miR-16 from total RNA (40 μg) isolated from day-8 to day-16 raft tissues derived from human foreskin keratinocytes with (HFK18) or without (HFK) HPV18 infection (A), or from day-10 rafts with or without HPV18 E6, E7, or E6E7 retrovirus infection (B). Northern blotting was also conducted to detect miR-16 expression in CaSki cells treated for 48 h with HPV16 E7–specific siRNA198 (40 nM) or in HeLa cells treated for 48 h with HPV18 E7-specific siRNA220 (40 nM) [121]). A nonspecific (NS) siRNA at the same dose was used as a control for both cells (C). U6 RNA was probed as internal loading control. Bar graphs on the right show relative miR-16 levels detected by Northern blot in each sample from a representative gel on the left after being normalized to U6 for sample loading.

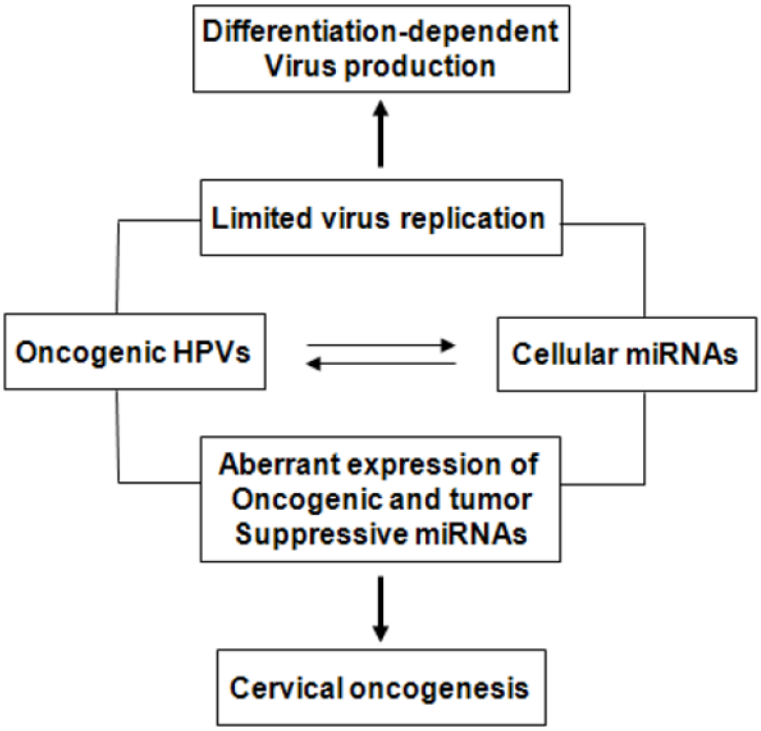

Fig. 6.
Outcomes of interactions between oncogenic HPVs and cellular miRNAs. These intimate interplays may lead to limited virus gene expression or uncontrolled cell proliferation by altering miRNA expression.

Table 1

Summary of miRNA expression profiling studies in cervical cancer

miRNA	Chr. Location	Wang et al[a]	Li et al[a]	Witten et al[b]
miR-15a	13q14.2	Up		
miR-15b	3q25.33	Up	Up	
miR-16	13q14.2	Up	Up	
miR-17-5p	13q31.3	Up	Up	
miR-20a	13q31.3	Up	Up	
miR-20b	Xq26.2	Up	Up	
miR-21	17q23.1	Up	Up	miR-21* Up
miR-93	7q22.1	Up	Up	
miR-106a	Xq26.2	Up	Up	
miR-146a	5q34	Up		
miR-148a	7p15.2	Up		
miR-155	21q21.3	Up	Up	Up
miR-181c	19p13.13	Up		
miR-182	7q32.2	Up	Up	
miR-183	7q32.2	Up		miR-183* Up
miR-185	22q11.21	Up	Up	
miR-223	Xq12	Up		
miR-224	Xq28	Up	Up	
miR-324-5p	17p13.1	Up		
miR-10b	2q31.1		Down	Down
miR-29a	7q32.3	Down	Down	
miR-30b	8q24.22	Down		
miR-34a[c]	1p36.22	Down	Down	
miR-125a	19q13.41	Down		
miR-125b	11q24.1		Down	Down
miR-126	9q34.3	Down	Down	Down
miR-127	14q32.2	Down	Down	
miR-133a	18q11.2	Down		
miR-133b	6p12.2	Down		
miR-143	5q32	Down		Down
miR-145	5q32	Down	Down	Down
miR-191*	3p21.31	Down		
miR-218	4p15.31;5q34	Down	Down	
miR-378(422b)	5q32	Down		
miR-422a	15q22.31	Down		
miR-424	Xq26.3	Down	Down	Down
miR-450	Xq26.3	Down	Down	Down
miR-455	9q32	Down	Down	
miR-574	4p14	Down		

Chr, chromosome; up, upregulated in cervical cancer; down, downregulated in cervical cancer.

[a] performed with an LC Science miRNA array platform [42;43].

[b] performed with a Solexa/Illumina sequencing platform [46].

[c] verified in separate studies [44;45].

Table 2

Alterations of cancer-associated microRNAs in cervical cancer

Oncogenic miRNAs	Tumor suppressive miRNAs
miR17-92 family	***let-7 family***
miR-21	miR-1
miR-26a	miR-9
miR-146a	***miR-15a/16-1 cluster***
miR-155	miR-26a
miR-205	***miR-29***
miR-221/222 cluster	***miR-34a/b/c***
miR-372/373 cluster	miR-101
	miR-124a
	miR-127
	miR-128
	miR-143/145 cluster
	miR-200/141 family
	miR-203
	miR-205
	miR-206/133b cluster
	miR-223

Oncogenic and tumor suppressive miRNAs, with the miRNAs altered in cervical cancer being bolded and italic. Summarized from [10;37;118].