Human papillomavirus (HPV) oncoproteins drive distinctive promoter methylation patterns in cancer. However, the underlying mechanism remains to be elucidated. Cyclin A1 (CCNA1) promoter methylation is strongly associated with HPV-associated cancer. CCNA1 methylation is found in HPV-associated cervical cancers, as well as in head and neck squamous cell cancer. Numerous pieces of evidence suggest that E7 may drive CCNA1 methylation. First, the CCNA1 promoter is methylated in HPV-positive epithelial lesions after transformation. Second, the CCNA1 promoter is methylated at a high level when HPV is integrated into the human genome. Finally, E7 has been shown to interact with DNA methyltransferase 1 (Dnmt1). Here, we sought to determine the mechanism by which E7 increases methylation in cervical cancer by using CCNA1 as a gene model. We investigated whether E7 induces CCNA1 promoter methylation, resulting in the loss of expression. Using both E7 knockdown and overexpression approaches in SiHa and C33a cells, our data showed that CCNA1 promoter methylation decreases with a corresponding increase in expression in E7-siRNA-transfected cells. By contrast, CCNA1 promoter methylation was augmented with a corresponding reduction in expression in E7-overexpressing cells. To confirm whether the binding of the E7–Dnmt1 complex to the CCNA1 promoter induced methylation and loss of expression, ChIP assays were carried out in E7-Δ, del CR3–E7 and vector control-overexpressing C33a cells. The data showed that E7 induced CCNA1 methylation by forming a complex with Dnmt1 at the CCNA1 promoter, resulting in the subsequent reduction of expression in cancers. It is interesting to further explore the genome-wide mechanism of E7 oncoprotein-mediated DNA methylation.

Cervical cancer is the third most common cancer in women worldwide. The prevalence of human papillomavirus (HPV)16/18 in cervical cancer cases worldwide is approximately 70%. Broadly speaking, the HPV genome is circular and comprises several genes; of these, the respective products (E6 and E7) of the oncogenic E6 and E7 genes have potent transforming capabilities, largely by targeting p53 and pRB, respectively, for degradation. In the integrated form, E6 and E7 become overexpressed due to E2 disruption. Hence, integrated HPV represents the major form of infection that is linked to tumorigenesis.

In addition to p53 and pRB degradation, data from recent studies have indicated that there may be additional mechanisms by which E6 and E7 contribute to cancer development. For instance, E7 may likely be involved in epigenetic alterations by augmenting the activity of DNA methyltransferase 1 (Dnmt1), resulting in a corresponding downregulation of E-cadherin that does not occur through promoter methylation. In another study by Hasan et al., E7 was shown to decrease H3K9 expression in cervical cancer, but this likely occurred through histone modifications. Recently, genome-wide methylation and expression studies indicated that HPV could increase the methylation and reduce the expression of several genes in head and neck cancer cells. A study by Sartor et al., showed the distinctive DNA methylation pattern due to HPV infection in HPV-positive and HPV-negative cell lines, including 84 formalin-fixed paraffin-embedded head and neck tumor samples. They observed that HPV-positive cells showed higher levels of promoter methylation than HPV-negative cells. Seventy-five genes were more highly methylated and expressed at lower levels in HPV-positive head and neck cancer samples compared with HPV-negative samples, including IRS1, GNA11, GNA12, EREG, CCNA1, RGS4, and PKIG. A study by Lechner et al. showed increased mRNA expression of both Dnmt3a and Dnmt1 in HPV-positive head and neck cancer cell lines. Moreover, they observed that gene methylation increased in E6- and E6 and E7-transfected head and neck cancer cell lines. Nevertheless, the mechanistic basis for this is not well understood. To explore the mechanism by which HPV induces increased methylation in HPV-associated cancers, we used CCNA1 as a gene model.

We have previously reported that methylation of the CCNA1 promoter was largely elevated in ~93% of cervical cancer cases, whereas the promoter remains unmethylated in normal
is now known to be prevalent in other solid tumors, such as breast cancer (27), indicating reduced expression and loss of function of the CCNA1 molecular marker for distinguishing between normal and cancerous tissue (23). One study revealed that the DNA methyltransferase activity of Dnmt1 (24). There are several mammalian members of the Dnmt family that display broadly similar functions, but some differences do exist. The Zinc finger CR3 region of E7 could interact with Dnmt1. Moreover, E7 can upregulate the expression of the gene product of CCNA1 in cervical cancer cell lines through a process that involves promoter methylation and Dnmt1.

Materials and Methods

Cell lines and culture. The human cervical carcinoma cell lines SiHa (HPV type 16-positive) (29) and C33a (HPV-negative) (29) were kindly provided by J. Silvio Gutkind (National Institutes of Health/NIDCR, Bethesda, MD, USA). They were grown and maintained in DMEM (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% FBS (Gibco, Carlsbad, CA, USA) and 1% antibiotic–antimycotic (Gibco Carlsbad, CA, USA) at 37°C in an atmosphere of 5% CO2.

5'-Azacytidine treatment. To evaluate methylation and expression changes, 5'-azacytidine (aza) (Sigma-Aldrich, MO, USA) was used to treat the cells. For treatment with aza, SiHa and C33a cells were seeded at 3 × 10^5 cells/mL in growth media. After overnight incubation, cells were further incubated with fresh media containing aza at the indicated final concentration (0–40 μM) for an additional 5 days, being replaced every 24 h until analysis.

Human papillomavirus type 16 E7 plasmid construction. To carry out transfection of the E7 recombinant plasmid in mammalian cells, plasmid 13634: pGEX2T E7 (Addgene, Cambridge, MA, USA), which expressed only in bacterial cells, was transformed to MAX Efficiency DH5α Competent Cells (Invitrogen, Carlsbad, CA, USA) for propagation. The mutagenesis of this plasmid (c.297A>T) was carried out using the QuikChange Lightning site-directed mutagenesis kit according to the manufacturer’s instructions (Stratagene, La Jolla, CA, USA). This mutated vector fragment was used as shown in Table 1. The PCR for the mutagenesis was carried out using the Q5 Site-Mutagenesis kit to mutate the stop codon TAA to TAT according to the manufacturer’s instructions of this plasmid (c.297A>T).

Table 1. Oligonucleotide sequences and conditions for PCR analyses

| Oligonucleotides       | Sequence (5’–3’) | Amplicon size, bp | Annealing temperature, °C |
|------------------------|-----------------|------------------|---------------------------|
| Primers                |                 |                  |                           |
| E7mutF                 | CCCATCTGTCTCAGAAAACATATGATGAATTCTCATGACTGG | 448 | 66 |
| E7mutR                 | CAGTCAAGATGAAATTCATCATATTGGGTTCTAGAAAGACATTGGG | 5,655 | 56 |
| E7del (F)              | GATGAATTCTGCGATATCC | 450 | 56 |
| E7del (R)              | TAATGGGCTCTGTCGGTTCT | 53 |
| GAPDH F                | GTGGGCAAGGTATCCTCG | 170 | 47 |
| GAPDH R                | GCCATCTGTTGAGGGGGAC | 142 | 56 |
| CCNA1metF              | TTTCAAGAGATTCCCGCTCGT | 46 | 53 |
| CCNA1metR              | CTCTTAAAACCCCTAACTGCA | 205 | 56 |
| CCNA1expF              | ATTCAATATGAAATTTGTC | 170 | 47 |
| CCNA1expR              | CTACCTACGAAATCTATTG | 142 | 56 |
| E7expF                 | GGGCATATTAAATGACGTCAG | 405 | 58 |
| E7expR                 | GTGTCCTTTGATGACCAACC | 205 | 56 |
| CCNA1-ChIPF            | CAGAAACCCGTAGTGTGAG | 405 | 58 |
| CCNA1-ChIPR            | GCTTTGGAAGGGGACCTTCCC | 405 | 58 |
| E-cadherin-ChIPF       | CGATCCCGGTCTTTAGTACC | 405 | 58 |
| E-cadherin-ChIPR       | GGCCTCAAGGGGCCATGCTGG | 405 | 58 |
| siRNA                  |                 |                  |                           |
| E7-Sense strand        | CAGAAACCCGTAGAAGGCGCAACUAAAC | 56 |
| E7-Antisense strand    | UUGUAUAUGGGCUCUGUGUGCCACUUCUG | 56 |
| Dnmt1-Sense            | UUAUGUUGUCUCAACAAUCUUCUG | 56 |
| Dnmt1-Antisense        | GACCAAGAGAUGUGUGAGCAGCAACUAAAC | 56 |

F, forward; R, reverse.
according to the manufacturer’s instructions (New England Biolabs, Ipswich, MA, USA). The primers used to introduce this deletion (forward and reverse) are shown in Table 1. The PCR for the mutagenesis was carried out according to the manufacturer’s instructions. The deletion of this region was confirmed by sequencing (data not shown).

**Transfection.** E7 overexpression was carried out in C33a cells, whereas the E7 and Dmnt1 siRNA experiments were carried out in SiHa cells. For E7 overexpression, C33a cells were seeded into six-well plates at 3 × 10⁵ cells/mL and incubated overnight. Next, 2 μg E7 recombinant plasmid and pC DNA 3.1/myc-HIS (PC) empty plasmid (Invitrogen) were transfected using Turbofect (Thermo Fisher Scientific) according to the manufacturer’s protocol. For the knockdown experiment, the E7 siRNA was designed using the BLOCK-iT RNAi designer online tool (accession number NC_001526.2). For Dmnt1 siRNA, the siRNA was designed following Zhang et al.,[30] and the sequences that were used for E7 and Dmnt1 are shown in Table 1. Prior to transfection, SiHa cells were first seeded at 5 × 10⁴ cells/mL in 12-well plates. Following overnight incubation, 40 pmol E7 and Dmnt1 siRNA and Stealth RNAi negative control sequences (Invitrogen) were transfected using Lipofectamine 2000 (Invitrogen). Next, 72 h after transfection, SiHa and C33a cells were collected to study E7 and CCNA1 mRNA expression, as well as CCNA1 promoter methylation.

**Isolation of DNA.** DNA was extracted from SiHAs and C33a cultures, digested with lysis buffer II containing SDS (Sigma-Aldrich) and proteinase K (USB, Cleveland, OH, USA) at 50°C overnight. Phenol/chloroform extraction and ethanol precipitation were then carried out as previously described.[33]

**Sodium bisulfite treatment and methylation-specific PCR.** DNA input of 750 ng for each sample was subjected to bisulfite treatment using the EZ DNA Methylation-Gold kit (Zymo Research, Irvine, CA, USA) according to the protocol provided by the manufacturer. The eluted DNA was consequently used to carry out methylation-specific PCR by using methylated and unmethylated specific primers (Table 1).[17] Then, 10 μL PCR products were observed by gel electrophoresis in 8% acrylamide and 1% agarose gel stained with SYBR (Lonza). The products were observed by gel electrophoresis in 8% acrylamide and 1% agarose gel stained with SYBR (Lonza). Next, the precipitated DNA was further analyzed by sequencing (data not shown).

**Western blot analysis.** Anti-E7 His tag (ab18184) Abcam (Cambridge, UK), Anti-Dnmt1 (ab13537) Abcam, and Anti-Dnmt3a (sc-20703) Santa Cruz Biotechnology (Santa Cruz, CA, USA) were obtained. Western blot analysis was carried out as previously described.[33] The total cellular protein concentrations of E7 protein (with or without CR3 region) and Dnmt1 were loaded for SDS-PAGE. The antibodies used for Western blotting are described in Table 2. All antibodies were differentiated by using the BLOCK-iT RNAi designer online tool (accession number NC_001526.2). For Dmnt1 siRNA, the siRNA was designed following Zhang et al.,[30] and the sequences that were used for E7 and Dmnt1 are shown in Table 1. Prior to transfection, SiHa cells were first seeded at 5 × 10⁴ cells/mL in 12-well plates. Following overnight incubation, 40 pmol E7 and Dmnt1 siRNA and Stealth RNAi negative control sequences (Invitrogen) were transfected using Lipofectamine 2000 (Invitrogen). Next, 72 h after transfection, SiHa and C33a cells were collected to study E7 and CCNA1 mRNA expression, as well as CCNA1 promoter methylation.

**ChIP analysis.** Anti-histone H3-trimethyl K4 (ab8580) Abcam, Anti-HPV16E7 (sc-6981) Santa Cruz Biotechnology, Anti-Dnmt1 (ab13537) Abcam, Anti-Dnmt3a (sc-20703) Santa Cruz Biotechnology, Anti-Dnmt3b (sc-20704) Santa Cruz Biotechnology, Mouse IgG isotype control (sc-2030) Santa Cruz Biotechnology, Mouse anti-HPV16E7 (sc-6981) Santa Cruz Biotechnology, Mouse anti-Dnmt1 (ab13537) Abcam, Mouse anti-Dnmt3a (sc-20703) Abcam, Mouse anti-Dnmt3b (sc-20704) Santa Cruz Biotechnology, Anti-Dnmt3a (sc-20703) 1:500 Santa Cruz Biotechnology, Anti-Dnmt1 (ab13537) 1:200 Abcam, Anti-HPV16E7 (sc-6981) Santa Cruz Biotechnology, Anti-Dnmt3a (sc-20703) 1:500 Abcam, Anti-Dnmt1 (ab13537) Abcam, and Anti-Dnmt3a (sc-20703) 1:500 Abcam, Anti-Dnmt1 (ab13537) Abcam, and Anti-Dnmt3a (sc-20703) 1:500 Abcam.

**Statistical analysis.** To test the two groups in each experiment, one-way ANOVA with repeated measures was used to determine statistical significance and level of P = 0.05 were considered significant.

| Antibodies for Western blot and ChIP analysis | Ratio | Company |
|----------------------------------------------|-------|---------|
| **Western blot analysis**                     |       |         |
| Anti-6x His tag (ab18184)                    | 1:2000| Abcam (Cambridge, UK) |
| Anti-Dnmt1 (ab13537)                         | 1:200 | Abcam   |
| Anti-Dnmt3a (sc-20703)                       | 1:500 | Santa Cruz Biotechnology (Santa Cruz, CA, USA) |
| Anti-Dnmt3b (sc-20704)                       | 1:500 | Santa Cruz Biotechnology |
| Goat anti-mouse IgG (HRP) (sc-2062)          | 1:10 000| Santa Cruz Biotechnology |
| Goat anti-rabbit IgG (HRP) (sc-2030)         | 1:10 000| Santa Cruz Biotechnology |
| Anti-β-actin (HRP) (ab49900)                 | 1:20 000| Abcam   |
| **ChIP analysis**                             |       |         |
| Anti-histone H3-trimethyl K4 (ab8580)        |       | Abcam   |
| Anti-HPV16E7 (sc-6981)                       |       | Santa Cruz Biotechnology |
| Anti-Dnmt1 (ab13537)                         |       | Abcam   |
| Anti-Dnmt3a (sc-20703)                       |       | Santa Cruz Biotechnology |
| Anti-Dnmt3b (sc-20704)                       |       | Santa Cruz Biotechnology |
| Mouse IgG isotype control (sc-2030)          |       | Cell Signaling Technology (Danvers, MA, USA) |
| Normal rabbit IgG (2729s)                    |       | Cell Signaling Technology |
| Dmnt, DNA methyltransferase.                 |       |         |
Results

CCNA1 promoter methylation associated with CCNA1 expression repression in cancer cells. Two cervical cancer cell lines, SiHa (HPV-positive) and C33a (HPV-negative), confirmed for their HPV status by using L1 primer (MY09/MY11), were subjected to CCNA1 promoter methylation and expression analysis. As shown in Figure 1a, SiHa cells showed full CCNA1 methylation, whereas only partial methylation (band intensity of 44.97%) was observed in C33a cells. For CCNA1 expression, we observed that SiHa cells had lower CCNA1 expression, giving a band intensity of 26.85%, compared with C33a (70.31%). We also used GAPDH as an internal control for normalization (Fig. 1b). The data suggest that the presence of HPV might influence CCNA1 promoter methylation and expression.

Lowering CCNA1 methylation increases CCNA1 expression. Next, we subjected C33a and SiHa cells to aza treatment to observe whether CCNA1 promoter methylation was involved in the control of expression of the product cyclin A1. The results showed that the band density of CCNA1 methylation in C33a cells gradually decreased with increasing aza concentrations (0–7 μM) while the unmethylated form of CCNA1 in C33a cells increased with corresponding aza treatment (data not shown). In parallel, the band density of CCNA1 methylation in SiHa decreased with increasing aza concentrations (0–40 μM). We observed that the methylation status of the CCNA1 promoter in C33a and SiHa cells decreased significantly with all aza treatment concentrations (P = 0.0005 and P < 0.0001, respectively) (Fig. 2a,b). CCNA1 expression was shown by quantitative RT-PCR and ΔΔCT, with the results revealing that expression continuously increased following aza treatment in C33a and SiHa cells. By one-way ANOVA, both cell lines showed significant increase in CCNA1 mRNA expression (P < 0.0001) (Fig. 2c,d).

Human papillomavirus E7 induces CCNA1 promoter methylation and decreases CCNA1 expression. To prove the effects of E7, we evaluated CCNA1 promoter methylation and mRNA expression changes after E7 expression reduction. Here, we used E7 siRNA in HPV-positive SiHa cells. We found that E7 siRNA-transfected SiHa cells had significantly less E7 mRNA expression (P = 0.0002) (Fig. 3a). CCNA1 promoter methylation was decreased in E7 siRNA-transfected SiHa cells compared with scrambled-transfected cells (P < 0.0001) (Fig. 3b). The levels of CCNA1 mRNA expression in E7 siRNA-transfected SiHa cells were increased compared with scrambled siRNA-transfected cells (P < 0.0001) (Fig. 3c). This observation revealed that the lower E7 mRNA expression in E7 siRNA-transfected cells decreased CCNA1 promoter methylation and increased CCNA1 mRNA expression.

Moreover, we evaluated whether E7 can de novo methylate the CCNA1 promoter using an E7 recombinant expression vector. 5-Azacytidine at 0, 3, 5, and 7 μM and 0, 20, 30, and 40 μM was used to treat C33a and SiHa cells, respectively, for 5 days, and cells were harvested to observe CCNA1 promoter methylation and expression. Eight replicates of the experiment were carried out. (a) Diagram and statistical test of the CCNA1 promoter methylation status in C33a cells. (b) Diagram and statistical test of the CCNA1 promoter methylation status in SiHa cells. (c) Fold change of CCNA1 expression in C33a cells. (d) Fold change of CCNA1 expression in SiHa cells.
The E7 recombinant expression plasmid and empty plasmid control (PC) were transfected into HPV-negative C33a cells. We observed that E7 gene expression was significantly elevated in E7 recombinant plasmid-transfected C33a cells compared with the PC control (P = 0.0036) (Fig. 4a), and a similar trend was observed for E7 protein expression (Fig. 4b). Furthermore, CCNA1 promoter methylation was significantly increased in E7-overexpressing C33a cells compared with control cells (P = 0.0072) (Fig. 4c), broadly suggesting an inverse correlation with CCNA1 mRNA expression (P = 0.0002) (Fig. 4d).

Expression of Dnmt1, Dnmt3a, and Dnmt3b in E7-transfected cells. To observe the expression of Dnmt1, Dnmt3a, and Dnmt3b in E7-transfected cells by Western blot analysis, a decreasing level of Dnmt1 protein in E7-overexpressing C33a cells was observed compared with control cells. In contrast, there was no difference between the expression of Dnmt3a and Dnmt3b in E7-overexpressing C33a and control cells (Fig. 5).

CCNA1 promoter methylation and expression in Dnmt1 siRNA-transfected SiHa cells. After knocking down Dnmt1, Dnmt1 mRNA expression was observed. The results showed its decreased expression (data not shown). Moreover, CCNA1 promoter methylation was decreased and its expression was increased significantly; P < 0.0001 and P = 0.0420, respectively (Fig. 6).

E7–Dnmt1 complex targets CCNA1 promoter. Because E7 promotes CCNA1 promoter methylation and is known to form a complex with Dnmt1, we hypothesized that E7 promotes CCNA1 methylation by forming an E7–Dnmt1–CCNA1 promoter complex. To demonstrate that our hypothesis was
viable, ChIP assays were carried out in C33a cells overexpressing E7, del CR3-E7, and PC control by precipitation with anti-HPV16 E7 antibody and then carrying out PCR to obtain a 205-bp CCNA1 product. As shown in Figure 7, the product band of the CCNA1 promoter was detected in E7, and del CR3-E7-overexpressing C33a cells, not in the control cells. Notably, by precipitating chromatin with the Dnmt1 antibody, a strong band was identified in the PCR product corresponding to CCNA1 in E7-overexpressing C33a but not in del CR3-E7 or control cells. There was no E-cadherin PCR band observed after precipitation with E7 and Dnmt1 in all transfected cells, confirming that E7-Dnmt1 binding was specific to the CCNA1 promoter, but not generally observed with other unmethylated promoters. Next, to investigate whether E7 had a preference for only Dnmt1 to methylate the CCNA1 promoter, we carried out ChIP–PCR using specific Dnmt1, Dnmt3a, and Dnmt3b antibodies for the immunoprecipitation. The results showed an intense, darker CCNA1 band following Dnmt1 precipitation in E7-overexpressing C33a cells compared with the control cells. There was no difference in the CCNA1 bands following Dnmt3b precipitation in both cells. However, a darker CCNA1 band was observed with Dnmt3a precipitation in the control cells compared with the E7-overexpressing C33a cells (Fig. 8). These data suggest that the E7-Dnmt1 complex can induce de novo methylation of CCNA1.

Discussion

We sought to determine the mechanism by which E7 changed the CCNA1 promoter methylation pattern in cervical cancer. We hypothesized that E7 might interact with Dnmt1 and thereby impact the status of CCNA1 promoter methylation with a consequent loss of CCNA1 expression in cervical cancer. To prove our hypothesis, we first investigated the methylation status in two cervical cancer cell lines, SiHa and C33a cells. We found that the CCNA1 promoter was fully methylated in SiHa cells and only partially methylated in C33a cells. It is possible that the stronger methylation band observed in SiHa cells might have come from E7 induction and, in contrast, the methylation in C33a cells might occur by spontaneous DNA methylation. There are several ways to control gene expression, including promoter methylation (18,35–38). Here, we provide evidence in our model system that promoter methylation is pivotal in controlling CCNA1 expression. After treatment of C33a and SiHa cells, decreased methylation was associated with increased CCNA1 expression. We can conclude that CCNA1 promoter methylation controls and regulates the expression of its gene product. Ultimately, we were able to show that E7 suppresses CCNA1 expression through CCNA1.
promoter methylation. Using ChiP assays, we detected the PCR product of CCNA1 after pulling down E7 and Dnmt1 antibodies in E7-overexpressing C33a cells compared with control cells. This implies that E7 and Dnmt1 formed a complex and bound to the CCNA1 promoter. This complex might be the cause of de novo methylation of the CCNA1 promoter in HPV-infected cells. Moreover, in support of our observations, recent data has shown that Dnmt1 is involved in CCNA1 promoter methylation and expression in SiHa cells.(39) Interestingly, we observed dark and intense PCR bands after precipitating Dnmt3a and Dnmt3b in both E7- and PC control-transfected cells. These results also relate to our prior observations, which showed that the CCNA1 promoter was methylated in untransfected C33a cells. Taken together, these results confirm the role of the E7–Dnmt1 complex in inducing CCNA1 promoter methylation.

The studies by Sartor et al. and Lechner et al.(15,16) confirm the impact of HPV-mediated promoter methylation of genes in HPV-associated cancers. However, the mechanism of virus-induced methylation was unknown. Here, we discovered this new mechanism using CCNA1 as a gene model. In addition to the induction of CCNA1 promoter methylation, there are several other methylated genes also induced by HPV. In addition, expression observation by mass spectrometry revealed evidence that E7 could upregulate and downregulate several genes in C33a cells transfected with HPV E7.(43) Moreover, E7 can bind to several transcription factors such as E2F, p300, and TBP.(40–42) Therefore, the proposed mechanism by which HPV induces methylation and suppresses expression of the genes is that the E7–Dnmt1 complex interacts with other transcription factors at gene promoters containing cis elements that respond to these transcription factors. This proposed mechanism requires further confirmation as to whether E7 can promote genome-wide methylation targeted genes.

There are many types of virus-associated cancers in addition to HPV-related cancers, including Epstein–Barr virus, and hepatitis B- and hepatitis C-associated cancers. There is evidence showing a correlation between the promoter methylation of genes and viral infection in these cancers.(43–45) A study by Kaneda et al. suggested that the overexpression of Dnmts by latent proteins and epigenomic changes, such as 3-D conformational changes and histone modifications, might be involved in the extensive induction of methylation in Epstein–Barr virus-associated cancer.(46) Nevertheless, there is no evidence revealing the mechanism by which methylation pattern changes occur due to viral carcinogenesis. Our study is the first to discover the mechanism of the increased methylation of genes due to viral infection.

In conclusion, this study revealed that E7 of HPV is able to induce CCNA1 promoter methylation by forming a complex with Dnmt1, resulting in decreasing expression of cyclin A1 mRNA. This finding provides a mechanistic model by which E7 of HPV mediates the methylation of several gene promoters in HPV-associated cancers. This present observation could be very useful for drug discovery in treating cancers associated with HPV infection, as well as many other diseases in the future, using an epigenetic treatment model.

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Disclosure Statement

The authors have no conflict of interest.

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