Epigenetic repression of E-cadherin by human papillomavirus 16 E7 protein

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A common feature shared between several human cancer-associated viruses, such as Epstein-Barr virus, Hepatitis B virus and Hepatitis C virus, and Human papillomavirus (HPV) is the ability to reduce the expression of cellular E-cadherin. Since E-cadherin is used by the host (14,15). Interestingly, numerous independent investigations have revealed that HPV-infected cervical epithelia possess fewer Langerhans cells than the uninfected neighbouring tissues, suggesting that HPV-infected cervical epithelia possess fewer Langerhans cells than the uninfected neighbouring tissues, suggesting that such epigenetic intervention clinically may be a way to re-establish the influx of Langerhans cells into infected epithelium to counteract HPV persistence.

Introduction

Infection of the cervical epithelium by high-risk human papillomaviruses (HPVs) can predispose this tissue to subsequent development of cancer (1,2). Activities of HPV proteins that can cause this have been elucidated and they include the degradation of p53 tumour suppressor protein, expression of the catalytic subunit of telomerase and inactivation or destruction of the retinoblastoma protein (pRb) protein. The first two activities are carried out by the viral E6 protein, whereas activities against the pRb are elicited by the E7 protein (3–7). While the potential contribution of E6 and E7 proteins to the development of cervical cancer is clear, infection by high-risk HPVs do not automatically result in cancers, as these viruses are usually cleared from the tissue after some time. Failure to clear the virus, however, would allow HPV to persist in the cervical epithelium and increase the likelihood of the eventual development of cervical cancer (8–11). Clearance of HPV may be elicited in part by the natural differentiation, migration and exfoliation of infected keratinocytes from the cervical epithelium. In addition to this, the host’s immune system also has an important role to play in viral clearance (12,13). Langerhans cells infiltrate and move within the epithelium and participate in detecting, processing and presenting foreign antigens to other immunocytes of the host (14,15). Interestingly, numerous independent investigations have revealed that HPV-infected cervical epithelia possess fewer Langerhans cells than the uninfected neighbouring tissues, suggesting that HPV creates a defined zone that is refractive to Langerhans cells influx within an otherwise healthy epithelium (16–27). Migration of Langerhans cells is influenced by cytokines such as tumour necrosis factor α (28,29), granulocyte-macrophage colony stimulating factor (30,31) and interleukin-10 (32) and chemokines including RANTES and MIP3α (33,34), which are produced by keratinocytes (35). HPV-containing cells were reported to have reduced expression of granulocyte-macrophage colony stimulating factor (31) and clinical lesions containing HPVs are reported to have altered levels of various cytokines which is unfavourable for the activation of the immune system (36). In addition to stimulatory signals, migration of Langerhans cells requires E-cadherin proteins to be present on the membrane of keratinocytes (21,29,37,38). Hence it is of particular significance that the amount of E-cadherin protein in HPV-infected tissues is significantly reduced (21,39–49) or its distribution altered (21,39–42,44,45,48–53).

Using the keratinocyte cell line NIKS, which was derived from human foreskin (54), we generated cells that harboured replicating HPV16 episomes that were able to persist in culture (reminiscent of a persistent infection) (55). Analyses of these cells revealed that the level of their E-cadherin protein was indeed reduced, as is seen in naturally infected cells of the cervical epithelium. However, cells that harboured HPV16 DNA mutant episomes, which do not express E7 protein (as a result of a stop codon within the E7 region) did not exhibit any significant reduction in the E-cadherin protein levels, demonstrating that it is the E7 protein that is predominantly responsible for this. The E7 protein does not target E-cadherin proteins for proteolytic degradation, and its ability to degrade pRb and AP-2α are not associated with E-cadherin reduction. Instead, the E7 protein augments the amount and activity of Dnmt1 in the cell, and this in turn causes a reduction in the transcription of the E-cadherin gene. These observations uncover yet another activity of HPV16 E7, which in this instance is one that works at the epigenetic level to contribute to the successful persistence of HPV in the infected epithelium. Importantly, inhibition of Dnmt activity re-established the level of E-cadherin expression of the host cell, suggesting that such an epigenetic intervention to recover E-cadherin expression may encourage re-infiltration of Langerhans cells back into HPV-infected regions of the epithelium.

Materials and methods

Cell culture

NIKS cells provided by Dr Paul Lambert were cultured in F-medium (three parts F-12 Ham:1 part Dulbecco’s modified Eagle’s medium, 5% foetal calf serum, 24 µg/ml Adenine, 8.4 ng/ml cholaer toxin, 5 µg/ml insulin, 0.4 µg/ml hydrocortison and 10 ng/ml epithelial growth factor). 3T3 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% foetal calf serum. Briefly, NIKS were grown in 10 cm plates containing 2 million gamma-irradiated J2-3T3 cells. To generate NIKS cells harbouring episomal HPV16 DNA, NIKS cells plated the day before at 0.5 million in a well of a six-well plate were transfected with 8 µg of recircularized HPV16 DNA and 0.2 µg of pCDNA6 (Invitrogen, Carlsbad, CA). The next day, the transfectants were passed into 10 cm plates with 2 million irradiated blasticidin-resistant J2-3T3. After attachment of cells on the plate, blasticidin was added into the medium to a final concentration of 8 µg/ml. Antibiotics selection was stopped when all the untransfected cells in the control plate were dead.

Plasmids, recircularization of HPV16 DNA and in vitro mutagenesis

The pSPW12 plasmid, from which recircularized HPV16 DNA was generated, was provided by Dr Margaret Stanley. Five micrograms of pSPW12 was digested with BamHI to release the full-length HPV16 DNA, followed by a ligation reaction with 2000 U of New England Biolab’s T4-DNA Ligase in

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a volume of 2 ml at 16°C overnight to recircularize the viral DNA. The recircularized DNA was purified and concentrated using the QIAGEN mini-prep kit according to the protocol provided. Mutagenesis to generate HPV16 DNA that cannot express the E7 protein was carried out using the Phu Ultra polymerase (Stratagene, La Jolla, CA) according to the protocol provided by the manufacturer. The mutagenesis primers used were forward primer ggttcagtatgatagtcacaag and reverse primer gttgtaaatataatattagcaatgt. They were used to introduce a stop codon at nucleotide position 595 of the HPV16 genome. The whole mutant viral RNA was sequenced to verify the integrity of the entire HPV16 sequence.

**Retrovirus production**

Retrovirus vectors (LXSN, LXSN16E6, LXSN16E7 and LXSN16E6/7) were kindly provided by Dr Denise Galloway. To produce retroviruses, these vectors were transfected into Phoenix A cells (kindly provided by Dr Nolan), and the medium of the cells harvested 48 h later and filtered through 0.2 μ filters. Aliquots were prepared and stored in −80°C until use. For infection, viruses were mixed with polybrene at a concentration of 10 μg/ml and layered onto the cell monolayer. Neomycin was used at 500 μg/ml concentration for selection.

**DNA extraction and Southern blotting**

DNA from cells was extracted using the QIAamp kit from QIAGEN (Valencia, CA) according to the protocol provided by the manufacturer. The eluted DNA was separated on a 1% agarose gel and blotted transferred to nylon filter and probed with 32P-labelled HPV16 DNA probe. After hybridization, the filter was washed and exposed to X-ray film or a phosphorimager screen and analysed.

**RNA extraction, reverse transcription and quantitative polymerase chain reaction**

J2-3T3 feeder cells were dislodged from the plate by forceful squirting with phosphate-buffered saline (PBS). Keratinocytes were trypsined and the cell pellet treated according to the protocol supplied by the RNeasy kit from QIAGEN. The quantity of the resulting nucleic acids were measured and 4 μg were transferred into a tube for DNase digestion according to the protocol supplied with the Ambion DNase away kit. The resulting RNA were reverse transcribed with Superscript polymerase (Invitrogen) and subjected to quantitative polymerase chain reaction (QPCR) analyses using primers to the E-cadherin complementary DNA sequence (5′-GGTTTCTCCCTACGTCTGTTGTTGGAGTA-GATGGGTTGTA-3′) and beta-actin complementary DNA (forward 5′TGGGATGGGATGGGATGGGATGGAAGAT and reverse 5′CGGCGAGGGCTAGAGAGGGA). The PCR product was separated on a 1% agarose gel and the expected DNA fragment of 272 bp was excised from the gel and purified using QIAquick Gel Extraction kit from QIAGEN according to the protocol provided by the manufacturer. The PCR products were then ligated into the pGEM-T Easy vector from Promega and transformed into XL1-Blue MRF- competent Cells from Stratagene. The cells were then plated onto agar containing ampicillin and X-gal over-night and single white colonies were picked for culture in Luria–Bertani broth containing ampicillin. QIAprep Spin Miniprep kit from QIAGEN was used to isolate the plasmid DNA. The DNA preparations were digested with EcoRI to release the cloned PCR fragment and separated on an agarose gel to verify fragment size. Individual plasmid clones obtained from NIKS and NIKS β were sent to Geneservice for sequencing using T7 primers. Secondary structure protocol sequencing was required in order to obtain complete sequences of the samples. The sequences were aligned using SeqMan software and all cytosines were analyzed for bisulphite conversion. Assuming that Cα, Cβ and Cτ (collectively referred to here as CpN) are not methylated, they are expected to be converted by the bisulphite reaction. Thus, each efficiency of the conversion reaction can be ascertained by providing the number of converted CpN by the total number of CpN in the sequence, and this gave conversion rates of 98.8% for NIKS and 99.3% for NIKS + HPV16 sequences. Furthermore, we ascertained that unconverted DNA (not put through the bisulphite reaction) could not be amplified with the primers used above. Conversely, primers designed to amplify unconverted DNA from the exact region of the E-cadherin promoter upstream that cannot amplify bisulphite converted DNA (data not shown) could not be amplified. The efficiency and completeness of the bisulphite conversion reaction is demonstrated by the fact that DNA sequencing of the converted and cloned PCR fragments revealed that virtually all (98.8 and 99.3%) the cytosines of CpN were converted to thymines in the individual clones. To control for the absence of over-conversion, we methylated the CpG of the DNA in vitro with SsII methyltransferase and then subjected it to bisulphite conversion in parallel with the test reactions and the protocol described above. Sequencing of these DNA revealed that while the cytosines of CpN were converted, those of CpGs were not (data not shown). Collectively, these controls demonstrate that the bisulphite conversion was efficient and specific (with no over-conversion).

**Results**

**Persistence of HPV16w1 episomes and HPV16E6/7-null episomes in NIKS cells**

In order to generate an in vitro culture system to study long term HPV persistence, NIKS cells, which were derived from foreskin
keratinocytes, were employed as they are capable of supporting stable
HPV DNA replication (54,55). HPV16 DNAs excised from plasmids
were recircularized and co-transfected into these cells with pcDNA6
plasmids, which express blasticidin-resistance protein. Following anti-
biotics selection, cells were passaged at least 10 times and at each
passage, DNAs were extracted from an aliquot of cells for analyses
by Southern blotting. As shown in Figure 1a HPV16 episomes persisted
in these cells without any apparent loss or gross reduction of copy
number through all the passages. In parallel, recircularized HPV16
genomes with a stop codon inserted in either the E6 or E7 open reading
frame were also put through the process described above. While the E6-
null HPV genome failed to persist (data not shown), the E7-null HPV16
genome readily persisted in these cells (Figure 1b). The unexplained
requirement for E6 in HPV16 persistence is consistent with similar
observations in several previous reports (57,58). These lines of cells
allowed us to address, in vitro, the above-mentioned phenomenon of
reduction of E-cadherin levels in HPV-containing epithelial lesions.

Differences in E-cadherin protein levels in NIKS, NIKS HPV16wt and
NIKS HPV16E7null cells
When protein lysates from NIKS cells and those that harboured wild-
type HPV16 episomes were analyzed, it was apparent that the level of
E-cadherin was significantly reduced in the latter, whether they were
in a state of confluence or not (Figure 2a). This is reminiscent of HPV-
containing cervical lesions, which were reported to have reduced
expression of E-cadherin (21,39,42,44,45,48,49). However, when ex-
tracts from two independently generated lines of NIKS cells bearing
HPV16E7-null episomes were tested, no appreciable reduction of the
E-cadherin levels was apparent (Figure 2b). Detection of E7 protein
eXclusively in cells harbouring the wild-type HPV genomes attests to
the fact that the E7-null mutant genomes were truly not expressing E7
proteins. The reduced levels of p53 protein in cells bearing wild-type
or E7-null viral DNA is indicative of E6 expression, as HPV16 E6 is
able to target p53 protein for proteasome-mediated degradation.
These analyses confirmed that wild-type HPV16 DNA expressed
both, the E6 and E7 proteins, whereas the HPV16 E7-null episomes
expressed only the E6 protein. As such, these results show that HPV16
E7 protein was responsible for the reduction of E-cadherin levels in
cells that harboured HPV16 episomes.

Fig. 1. Southern blot analyses of DNA extracted from various passages
(p) of (a) NIKS cells that harbour wild-type HPV16 DNA and (b) NIKS cells
that harbour E7null HPV16 episomes. The blots were probed with full-length
HPV16 DNA. The size marker used was a 1 kb supercoiled DNA ladder.

(a) Confluent

NIKS + HPV16wt

p 2 3 4 5 6 7 8 9 10 11

8kb

(b) Confluent

NIKS + HPV16E7null

p 2 3 4 6 7 8 9 10

8kb

Fig. 2. (a) Western blot of proteins extracted from NIKS and NIKS-bearing
epislomal HPV16 DNA. Lysates were prepared from either confluent or
subconfluent cells. The membrane was probed with antibodies against
E-cadherin or actin. (b) Lysates of NIKS cells, NIKS carrying wild-type
epislomal HPV16 DNA and two independently derived NIKS lines
harbouring E7null HPV16 episomes were analysed by western blotting using
antibodies against HPV16 E7, p53, E-cadherin and actin. Two independent
analyses of E-cadherin of these cells are shown. (c) Western blots of protein
lysates from NIKS cells that were infected with LXSN retrovirus (vector),
LXSN E6, LXSN E7 or LXSN E6/7, after selection with neomycin.
Antibodies against E-cadherin, p53 and actin were used.
HPV16 E7 alone is responsible for the reduction of E-cadherin protein level in cells

While the evidence is sufficiently compelling to suggest that the E7 protein is responsible for the reduction of E-cadherin protein level, it does not exclude the possibility that the E6 protein might also be required for this activity. This possibility is particularly pertinent in view of a report that demonstrated HPV16 E6 to reduce E-cadherin levels (41). In this light, it is conceivable that E6 and E7 proteins may act together to bring about this effect. To test this, NIKS cells were infected with retroviruses bearing E6, E7 or E6 plus E7 (E6/7) genes. Infected cells were selected with neomycin and the surviving infectants were harvested and their E-cadherin proteins analyzed (Figure 2c). The E-cadherin level in E6-expressing cells was comparable with that of control cells. However, E7 and E6/7 infectants exhibited markedly reduced levels of E-cadherin protein. Consistent with what has been reported regarding the effects of E6 and E7 proteins on p53; the p53 protein level in E6-expressing cells was reduced (5), whereas that in E7-expressing cells was increased (59). These results show that HPV16 E7 alone is able to reduce E-cadherin protein levels, and the absence or presence of E6 has no perceivable impact on this activity. It must be noted that in the multiple repeats of these experiments, there were occasions when the E6 protein alone was seen to slightly reduce E-cadherin protein level. However, unlike E7-induced reduction of E-cadherin, the E6-induced reduction is modest and curiously inconsistent. This erratic effect of E6 is apparent in some results in the following sections below.

HPV16 E7 protein does not target E-cadherin protein for degradation

To ascertain how HPV16 reduces E-cadherin level, we first tested the possibility that E-cadherin may be targeted for proteasomal degradation. Cells were either mock treated or treated with proteasome inhibitor ALLN, and the proteins from these cells were analyzed by western blotting (Figure 3a). While the E-cadherin protein levels in NIKS cells were augmented upon proteasome inhibition, E-cadherin protein level in cells that harboured HPV16 DNA was not altered. That the proteasome was indeed inhibited was attested by the fact that ALLN treatment increased the p53 protein levels in all the cells as p53’s degradation is proteasome mediated. This result suggests that HPV16 does not reduce E-cadherin levels in cells by channelling E-cadherin protein for degradation by proteasome.

HPV16 E7 suppresses transcription of E-cadherin gene

We set out to test the alternative possibility that HPV16 may repress the expression of the E-cadherin gene instead. RNA isolated from NIKS cells, NIKS bearing HPV16 DNA and NIKS cells infected either with control or E7 retroviral vectors were reverse transcribed and the complementary DNA of E-cadherin and that of beta-actin were measured using quantitative PCR. The results in Figure 3b show that the amount of E-cadherin transcripts (normalized against beta-actin transcripts) in cells harbouring HPV16 episomes or expressing E7 was markedly reduced. Although this result does not exclude the possible involvement of non-proteasomal degradation of E-cadherin, it nevertheless demonstrates that repression of gene expression is a means by which HPV16 reduces E-cadherin levels in the cell.

Reduction of E-cadherin protein level by HPV16 E7 is independent of pRb degradation or AP-2a downregulation

The best-characterized activity of HPV16 E7 is pRb degradation. As pRb protein has a profound effect on the expression of many cellular genes, it is possible that E7’s repressive effect on E-cadherin expression is mediated via pRb degradation. Indeed, a recent report alluded to the possibility that degradation of pRb and AP-2a is responsible for repressing E-cadherin expression (60). Immunoblotting with antibodies against pRb and AP-2a revealed that while pRb level was clearly reduced in HPV16-containing cells, the level of AP-2a protein remained unchanged between NIKS and NIKS + HPV16wt, in spite of a very clear reduction of E-cadherin level in the latter cells (Figure 4a). This observation excludes AP-2a degradation as a means by which E7 represses E-cadherin expression in our experimental system. To test whether pRb degradation by itself could reduce E-cadherin levels, we infected NIKS cells with recombinant retroviruses expressing short hairpin RNA against pRb. From Figure 4b, it is clear that although short hairpin RNA against pRb reduced the pRb protein level markedly, the level of E-cadherin was unchanged, indicating that degradation of pRb is not the means by which E7 represses E-cadherin expression.

HPV16 E7 augments the level of Dnmt1 in cells

In considering how E-cadherin expression can be suppressed by HPV E7, it is of particular interest to note how other viruses affect similar repression on the expression of this gene. In particular, the hepatitis B virus (HBV) also reduces E-cadherin expression in cells (61–63). This is achieved by the HBV X protein, which augments the level and activity of Dnmt1, which goes on to repress E-cadherin expression. As such we considered the possibility that the HPV16 E7 may also do likewise. Analyses of the Dnmt1 protein of NIKS cells and those that harbour HPV16 episomes revealed that HPV16 does indeed augment greatly the level of Dnmt1 (Figure 4c). Dnmt3a level was also
marginallly increased, whereas the presence of Dnmt3b and Dnmt3L were below the level of detection (data not shown). By immunoblotting NIKS cells that were infected with either empty retroviral construct or those bearing the E6 or E7 genes, we observed that the E7 protein increased greatly the protein level of Dnmt1 (Figure 4d). To test whether the augmented levels of Dnmt1 protein affects Dnmt activity, we assayed the lysates of these cells and observed that HPV16 or HPV16E7 protein alone, both increased the activity of Dnmt in the cells (as shown in Figure 4e).

Inhibition of Dnmt activity allows recovery of E-cadherin levels

Augmentation of Dnmt activity by HPV16 is particularly relevant in the context of repression of E-cadherin expression. Many cellular promoters, including that of E-cadherin, are silenced when methylated by Dnmts. Indeed it is through such a mode of repression that E-cadherin levels are diminished by HBV (61–63), Hepatitis C virus (HCV) (64,65) and Epstein-Barr virus (EBV) (66–69). If augmenting Dnmt activity is relevant to HPV-mediated repression of E-cadherin, then inhibition of Dnmt would be expected to derepress E-cadherin expression in cells containing HPV DNA or HPV oncogenes. To test this, 5-aza-deoxycytidine or DAC, an inhibitor of Dnmt was fed to the cells. This resulted in the recovery of the E-cadherin protein levels in cells containing HPV16 DNA or expressing E7 protein alone, both increased the activity of Dnmt in the cells (as shown in Figure 4e).

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E-cadherin was measured (Figure 5b). Importantly, cells devoid of HPV or its oncogenes were refractive to 5-aza-deoxycytidine, confirming that the HPV-induced augmentation of Dnmt activity does indeed cause the repression of E-cadherin expression. The observations described hitherto demonstrate that HPV increases cellular Dnmt activity and this in turn is responsible for decreasing the E-cadherin gene expression. It does not, however, mean that Dnmt acts directly on the E-cadherin promoter. To test this, we subjected DNA from NIKS and NIKS + HPV16 to bisulphite reaction which converts cytosines to thymines, unless the cytosines were methylated. This was followed by PCR cloning and sequencing of the E-cadherin promoter. We observed that NIKS cells harbouring HPV16 episomes did not exhibit any convincing differences in the CpG methylation state of their E-cadherin promoters from those of cells devoid of the virus. In fact the E-cadherin promoters in these cells (with or without HPV16) were almost fully unmethylated (Figure 6). This observation means that while HPV-induced augmentation of Dnmt activity is responsible for repressing E-cadherin gene expression, this repression is not brought about by methylation of the E-cadherin promoter. Instead, the results suggest that the E7-increased Dnmt activity targets a cellular genes whose protein products impinge on the E-cadherin promoter. One potential candidate is Slug, the repressor of E-cadherin expression (70,71). We tested to see if the protein level of Slug is altered in these cells. Western blotting revealed that the level of Slug was comparable in all the cells tested (data not shown), hence excluding it as the mediator of E-cadherin repression by E7. At this point in time, we do not know the Dnmt target that is responsible for the HPV-induced reduction of E-cadherin. This notwithstanding, it is clear that HPV, like the other cancer-associated viruses (HBV, HCV and EBV) is able to modulate through epigenetic means, expression of the cellular E-cadherin gene. While this results in direct methylation of the E-cadherin promoter by the other viruses, HPV-induced augmentation of Dnmt activity results in the indirect repression of the E-cadherin promoter.

Discussion

Observations from numerous independent analyses of clinical samples have consistently revealed that HPV-containing lesions have markedly reduced levels of E-cadherin protein. This study and those of Matthews et al. (41), Caberg et al. (60) and Hellner et al. (72) collectively show that this is not a secondary feature that is acquired by the cell independently of HPV action. Instead HPV directly reduces cellular E-cadherin expression. In the experiments described above, we show that the E7 protein is capable of reducing E-cadherin levels independently of any other viral proteins. As such we conclude that the E7 protein is the major instigator of E-cadherin reduction. However, it is noteworthy that the E6 protein may also contribute to this activity in vivo. We are mindful that in the context of the tissue, the relative expression and contribution of E6 and E7 to reducing E-cadherin level may be different or synergistic. This notwithstanding, it is clear that the E7 protein is able to do this on its own as expression of E7 alone or in the context of E6 resulted in comparable reduction of E-cadherin. While it would have been ideal to generate a NIKS line that harbour HPV16E6null DNA, we and others have not been able to generate such a cell line beyond the second passage (highlighting the requirement of E6 for HPV persistence in replicating cells) (57,58). Hence recombinant retroviruses were used to address this question instead. Although we can be confident that reduction of E-cadherin is not brought about via degradation by the proteasome system, we cannot exclude the possibility that E-cadherin could be channelled by E7 for degradation via another cellular proteolytic system. What is clear, however, is that E7 does reduce the expression of E-cadherin at the transcription level as the quantity of E-cadherin RNA was clearly reduced in cells with HPV16 DNA or HPV16E7 protein. The report by Caberg et al. (60) suggests that E7 does this by the combined reduction of pRb and AP-2α. From the outset, this did not appear to be case in our cells as while pRb was reduced by E7, the
level of AP-2 was not. This is consistent with a previous report that showed that although reduction of AP-2x can occur, as it does in SiHa cells used by Caberg et al., it is actually a rare event in cervical cancers (73). Furthermore, knock down of pRb levels using shRNA did not reduce E-cadherin levels. Our attention was turned to the interesting fact that several other cancer-associated viruses (HBV, HCV and EBV) also reduce E-cadherin level by reducing expression of this gene. They all did this by repressing the E-cadherin promoter. HBV, via its X protein, activates Dnmt1 expression and causes the repression of E-cadherin expression (61–63). Likewise, EBV latent membrane protein 1 protein activates Dnmt1 expression via C-Jun-N-terminal kinase-Activator protein 1 pathway and as a consequence represses E-cadherin expression (66–69). HCV core protein represses E-cadherin also by activating Dnmt1 and Dnmt3b (64,65). It is very likely that these viruses (including HPV) derive the common advantage of immune evasion by this method. Interestingly all these viruses (HBV, HCV and EBV) including HPV16 repress E-cadherin expression via activation of Dnmt1. For HPV16, this was brought about by E7-mediated augmentation of Dnmt1 protein and activity. This characteristic of E7 was also observed by Burgers et al. (74), who showed that E7 associates directly and physically with the Dnmt1 protein. This association contributes to the stability of the Dnmt1 protein (74). Mutation of pRb-interaction region of the E7 protein did not obliterate the ability of E7 to activate Dnmt activity, demonstrating that E7 increases the levels and activity of Dnmt primarily by direct association with the protein (74) and independently of pRb degradation as observed by us. The relevance of Dnmt1 increase and activation in regards to E-cadherin repression was made clear when inhibition of Dnmt by 5-aza-deoxycytidine re-established RNA and protein levels of E-cadherin in cells containing HPV16 DNA or expressing HPV proteins but not in control cells that do not contain HPV DNA.

Up to this point, the mechanism by which HPV16 repressed E-cadherin appears to parallel those of HBV, HCV and EBV. However, while the other viruses instigated a Dnmt1-mediated methylation of the E-cadherin promoter, neither HPV16 nor E7 protein appeared to do this. Instead the promoter of E-cadherin remained largely unmethylated whether or not HPV was present. This suggests that E7-augmented Dnmt1 activity is targeted to a cellular gene/genes whose protein acts on the E-cadherin promoter. Testing revealed that although the protein Slug was a prime candidate to be such a mediator (71,75;76), neither its level nor localization was altered by E7. As yet we have not identified what the cellular factors are. The difference between HPV and the other cancer-associated viruses is interesting when we consider that while cancers (hepatocellular carcinomas and lymphomas) that are associated with the other viruses (HBV, HCV and EBV) usually have their p16 promoter repressed by DNA methylation (HPV16 containing) cells in an organotypic culture. As such the induced migration of Langerhans cells into stratified layers of SiHa (HPV16 containing) cells in an organotypic culture. As such the induced migration of Langerhans cells into stratified layers of SiHa (HPV16 containing) cells in an organotypic culture.

Conflict of Interest Statement: None declared.

Acknowledgements

We would like to thank all the members of the lab for their assistance. We are grateful to Jonathan Stoye for support and encouragement.

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Received August 27, 2009; revised January 13, 2010; accepted January 24, 2010.