Papillomavirus E5 Proteins

Aaron L. Halpern[a] **and Dennis J. McCance**[b]

[a] *Theoretical Biology and Biophysics, T10, MS K710, Los Alamos National Laboratory, Los Alamos NM, 87545 USA*
[b] *Department of Microbiology and Immunology, University of Rochester, Box 672, 601 Elmwood Avenue, Rochester, NY 14642 USA*

Contents of This Chapter

This document is divided into three parts, with associated tables at the end. **Part I** reviews the E5 protein of bovine papillomavirus type 1 (BPV1). **Part II** reviews the E5(a) proteins of the mucosal papillomaviruses, specifically HPV16, HPV6b, HPV11 and rhesus papillomavirus (RhPV). **Part III** briefly discusses other PV proteins to which the name "E5" has been applied, as well as the E8 protein of BPV4.

I. The E5 Proteins of BPV1

BPV1 E5 is a 44 amino acid, membrane-associated protein which is the primary transforming protein of BPV1 [6, 19, 31, 65, 68, 69]. It migrates as an approximately 7 kd monomer in reducing conditions and as a 14 kd dimer in neutral conditions [69, 6], apparently due to disulfide bonds between monomers. There is no evidence of posttranslational modifications of E5, e.g., phosphorylation [29].

Its small size has led to the hypothesis that E5 acts by modifying the activity of cellular proteins rather than through direct enzymatic activity [26, 29], which is supported by evidence that it forms a complex with various cellular proteins. E5 has been shown to interact with the β-type platelet-derived growth factor receptor (β PDGF-R) [53], and, less clearly, the epidermal growth factor receptor (EGF-R) [14], as well as a 16 kd membrane protein, 16K, which forms part of the vacuolar H^+-ATPase [26].

In contrast to the transforming functions of E6 and E7, which seem to involve interference with pathways which normally suppress growth, those of E5 seem to involve activation of growth inducing pathways [53]. Some groups have found that BPV1 E6 is needed in addition to E5 for sustained growth [9, 52].

Transformation by E5 has been established in a number of systems, either in the context of a complete BPV genome (with frameshift knockouts of E6 and E7) or under the control of a variety of other heterologous promotors. BPV1 E5 alone is sufficient to induce focus formation, although E5 knockouts indicate that E6 is weakly transforming by the same assay [52]. Overexpression of E5 in rodent fibroblast cell lines leads to induction of DNA synthesis [1, 30, 41, 42, 66, 71], morphological transformation [19, 42, 54, 68, 70, 71], focus formation and anchorage independent growth [6, 33, 34, 42, 50, 54, 71, 72, 73], and tumorigenicity in nude mice [53], as well as similar behavior in keratinocyte lines [44]. Cooperation between E5 and *ras* in transforming NIH3T3 cells has been shown [80].

In naturally infected tissues, E5 is seen in two distinct cell types; it is present in basal keratinocytes at low levels [3, 8], possibly leading to a sustained transformed state of these cells, and in highly differentiated keratinocytes at higher levels with a granular staining pattern [8]. In the differentiated cells, E5 is in close association with capsid proteins, possibly indicating a role in viral maturation such as the induction of DNA synthesis during vegetative replication [3]. The E5 in the basal and upper epithelial cells may be coded for by different mRNAs [3].

Many of the biological activities identified for BPV1 E5 are likely to extend to the E5 proteins of the related BPV2, BPV5, deer papillomavirus (DPV), European elk papillomavirus (EEPV) and ovine papillomavirus (OvPV), all of which have extremely similar sequences.

Summary table for BPV1 E5 studies

BPV1 E5	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF
Focus Formation	--nlwfllfl glv-AMQLL- L-f----flv YWDhfeCsC- ----
DNA Synthesis	---------- -----aMQLL- llF----[--V YWDhFeC-C- g---]
Dimerization	--nlwfllfl glv-a-Q--- ll-------V -Wd-F-C-C- ----
Morphological Transformation	---------- ----a-QLL- Ll-------V yWDhFeC-C- g---
16K Binding	[---------- ----a-Q--- llf-------] -wdhfec-c- g---
PDGF-R Binding and Phosphor.	[---------- ------Q--- ---------v] -wDhfeC-C- ----
PDGF-R Downregulation	---------- ---------- ---------V -WD-F-C-C- ----

Summary of mutational studies of BPV1 E5: The 44 amino acid E5 protein has been extensively studied by examining the functionality of mutated proteins. The first line of the figure gives the sequence of E5 for the reference strain [10]. The following lines each summarize the mutations which have been assayed for retention of a given property. Positions which have been mutated are shown in one of three states: a lower-case letter ("a") indicates a position which has been mutated with little or no loss of function; a letter in white on gray ("A") indicates a position which has been mutated with moderate loss of function; a letter in white on black ("A") indicates a position which has been mutated with substantial or total loss of function. Positions which have not been mutated for a given assay are indicated as dashes ("-"). Boxes around portions of the sequence indicate regions which are sufficient on their own to induce the indicated activity to near-wild-type levels.

Details regarding the various activities of BPV1 E5 are given in the following sections; tables summarizing mutational studies of the relevant biological activities appear at the end of the chapter. An overall summary of the mutational studies is given in the table immediately above.

I.A. Localization, Expression Levels, Stability, and Mobility

Examination of the sequence of E5 reveals that residues 1–30 are generally hydrophobic (with the notable exception of Q17), compatible with the predominant localization of E5 in cell membranes. E5 is found in the endoplasmic reticulum and golgi apparatus, and to a lesser degree in the extracellular membrane, with the mostly hydrophilic C-terminus (aa 31-44) oriented away from the cytoplasm [7]. One study found localization to the distal golgi necessary to stimulate mitotic signaling and transformation [73].

The mutational studies summarized below have generally shown little evidence of changes in localization, expression levels, stability, or electrophoretic migration as a consequence of specific

mutations, although occasional differences have been noted (e.g., reduced membrane association of certain mutations [33], reduced levels of protein [71], or altered mobility [6]).

I.B. Focus Formation

Transfection with BPV1 E5 leads to focus formation and anchorage independent growth in a variety of cell lines, including rodent fibroblast or fibroblast-like cells (NIH3T3 and C127) [6, 33, 34, 42, 50, 54, 71, 72, 73], rodent epithelial cells (p117 and Pam212) [44], normal mouse mammary gland cells (NMuMG) [53], and bovine conjunctival fibroblasts [8]. This transforming activity may be augmented by treatment of cells with PDGF or EGF, or cotransfection with PDGF-R, EGF-R, CSF-1-R [46] or insulin-like growth factor receptor [51]. Mutations which affect homodimerization or the formation of complexes between E5 and PDGF-R or 16K, may reduce or eliminate transforming activity [54, 72, 73].

Mutations to the central portion of the protein which replace one hydrophobic residue with another do not greatly affect transformation, while replacement of hydrophobic residues with polar or charged residues reduces or eliminates transformation [33, 42, 50, 72]. Replacement of Q17 gives a wide range of effects, from complete elimination of transformation to augmented activity [72]; certain changes appear to give markedly different results in C127 and NIH3T3 cells e.g. Q17D, where this mutation transforms C127 but not NIH 3T3 cells [72]. The entire central portion of E5 (aa 14–29) can be replaced by certain "random" hydrophobic sequences without great loss of activity, suggesting that there are few if any crucial sequence-specific contacts in this region [34]; inclusion of an analog of Q17 rescues several otherwise defective proteins, but not all [42, 50].

In the C-terminus, mutations of Y31, W32, D33 and the dimerizing cysteines C37 and C39 have the largest effects, although other residues reduce focus formation too [33, 34, 50, 71]. Mutations progressively removing additional residues from the C-terminus and changing the final residue of the resulting chain to Cys identify D33 plus a final cysteine as the minimal C-terminus for efficient tranformation, although this series of mutations also involved a Q17E substitution [50].

Mutational studies suggest little role for the N-terminal portion of the molecule [6].

➢ See **Table I.1** for a summary of the effect of various mutations on focus formation.

I.C. Morphological Transformation

Transfection of BPV1 E5 into C127 or NIH3T3 cells leads to morphological transformation [19, 68]. Transfection of high levels of a plasmid containing E5 under the control of the SV40 early promotor leads to an acute morphological transformation [42, 54, 70, 71]. Mutations affecting this transformation are largely as discussed for DNA synthesis induction (although nearly all mutations lead to some degree of morphological transformation), and are also fairly tightly correlated with effect on focus formation [71]. Certain cell lines (mouse keratinocyte p117) transfected with E5 did not undergo easily detectable morphological transformation, nor focus formation, but did lead to tumors when injected into nude mice [44].

➢ See **Table I.2** for a summary of the effect of various mutations on morphological transformation.

I.D. DNA Synthesis

Both injection of synthetic E5 C-terminal fragments into the nucleus (or, less efficiently, the cytoplasm) and transfection of E5 lead to DNA synthesis in previously quiescent, serum-starved C127 cells [1, 30, 42, 66, 71]. Under control of BPV1 regulatory elements, deer papillomavirus (DPV) E5 can likewise induce DNA synthesis [41]. Cells undergoing DNA synthesis are primarily in a 4n state, suggesting a block in G2, the premitotic phase of the cell cycle [1], and indicating a possible function for the high levels of E5 expression observed in differentiated cells, namely an involvement in vegetative replication [3].

Mutations of E5 reveal a fairly tight correlation between induction of DNA synthesis, acute morphological transformation, and focus formation, but not necessarily dimerization [42, 71]. Mutation of Q17 to L or G, introduction of non-hydrophobic residues into the hydrophobic domain, mutations

V30L+F35S, W32S, or D33V, and mutation of either or both of C37S and C39S substantially or completely reduce DNA synthesis induction. However, only mutations C37S and C39S affect dimerization. Many, but not all, random peptides which retain a residue analogous to Q17 and are otherwise hydrophobic may functionally replace the central portion of E5, and certain peptides may omit the Q17 analog and still induce DNA synthesis [42].

➢ See **Table I.3** for a summary of the effect of various mutations on DNA synthesis induction.

I.E. Dimerization

BPV1 E5 migrates as an approximately 14 kd dimeric band under nonreducing conditions, and as an approximately 7 kd monomeric band under reducing conditions. Bands presumably corresponding to larger oligomers (trimers, etc) have also been observed [6]. Dimerization of E5 suggests that one of its functions may be to dimerize bound monomeric receptors [25].

Mutations of C37 or C39 reduce dimerization, while mutation of both leads to little or no dimerization. Mutations to the N-terminal and central portions of E5, including Q17, generally appear to have little effect on dimerization [27, 72], but inclusion of a Q17-analog in random hydrophobic segments substituted for the middle of E5 does seem to be necessary for extensive dimer formation of these mutations [42]; the reason for this discrepancy is unclear.

➢ See **Table I.4** for a summary of the effect of various mutations on dimerization.

I.F. PDGF Receptor and PDGF

Several lines of evidence indicate the importance of interactions between E5 and the β-type platelet-derived growth factor receptor (β PDGF-R). The two are observed to form a complex involving either endogenous or transfected β PDGF-R [14, 20, 25, 28, 53, 54, 63, 74]; transfection of E5 leads to the same patterns of β PDGF-R phosphorylation, tyrosine kinase activity, induction of DNA synthesis, and focus formation in C127 cells as are observed upon treatment with PDGF [63]; cell lines which do not respond to E5 alone and do not express significant levels of endogenous β PDGF-R may be transformed by cotransfection of E5 and β PDGF-R [28, 53]; cell lines which show abnormal response (reduction of DNA synthesis) to treatment with PDGF show the same abnormal response to E5 [67]; mutations which interfere with E5/β PDGF-R interaction (complex formation, receptor phosphorylation, kinase activity, or receptor-down regulation) may be deficient in tranforming activity as well [25, 54]. Interaction of E5 and β PDGF-R is specific: functional interaction is not seen with related α PDGF-R; and complexes between E5 and EGF-R, CSF-1-R, and α PDGF-R are either not observed or are disfavored under conditions where the E5+β PDGF-R complex is present [28, 62]. It should however be noted that E5 can transform cells which do not express detectable levels of β PDGF-R, suggesting that this interaction is not the only function of E5 [44].

Sequence similarity between the C-terminal portion of E5 and the receptor ligand PDGF led to early speculations of a common binding site [63], but subsequent studies indicate that this is unlikely since: the relevant portion of PDGF is not involved in binding the receptor [57]; the extracellular, ligand-binding portion of PDGF-R is not necessary for its interaction with E5 [14, 20, 74]; mutations of the apparently analogous residues of E5 and PDGF have different effects [45]; and deletion of the entire C-terminal, potentially extracellular portion of E5 does not eliminate, although it does reduce, association with the receptor [25].

Similarity between PDGF and E5 [45, 63]

Protein	Sequence	aa
PDGF A	EHLECTCT	
v-sis/PDGF B	FLV ... DHLACKCE	148–150,204–210
BPV1 E5	FLV ... DHFECSCT	28–30,33–40
DPV E5	DQFGCKCE	

Construction of chimeric receptors indicates that both the transmembrane and juxtamembrane portions of PDGF-R are involved in a complex with E5 [20, 74]. The importance of E5 D33 and the presence of β PDGF-R K531 just outside the cellular membrane suggests a possible salt bridge [74]. Although it has been suggested that association between E5 and β PDGF-R is due to each of the proteins binding to 16K (see section G) [25], the differences in the effect of mutations to E5 D33 and C37/C39 on binding to 16K [27] and binding to PDGF-R [54] raise questions. Both mutations bind 16K but not the β PDGF receptor.

E5 association with β PDGF-R does not appear to increase receptor half-life or receptor levels, in contrast to EGF-R, but rather to increase autophosphorylation of the receptor and receptor tyrosine kinase activity [25, 46, 63]. Indeed, overexpression of E5 at least in some systems appears to lead to *down*regulation of receptor [25, 54]. Neither autophosphorylation nor kinase activity alone appears to be sufficient for transformation [20, 74], but the specific roles of these activities are poorly understood.

Intriguingly, it appears that E5 binds primarily to the immature, intracellular form of β PDGF-R, which is consequently phosphorylated [53, 61], although complexes between E5 and the mature β PDGF-R have also been observed [25]. Whether activation of this immature form is important or is just the mechanism by which the mature form, located in the extracellular membrane away from the majority of E5, is activated is unclear; see [63] for discussion.

➢ See **Table I.5.a, I.5.b and I.5.c** for a summary of the effect of various mutations on binding, upregulation and phosphorylation of the β PDGF-R.

I.G. 16K

BPV1 E5 forms a complex with a 16 kd protein, 16K (also known as ductin), which has been identified as a subunit of the vacuolar H^+-ATPase [26, 29]. Direct binding of E5 and 16K has been proposed on the basis of high levels of complex when the two proteins are overexpressed [25], with the apparent molecular weight of the complex suggesting that dimeric E5 binds to two 16K molecules [27]. 16K has also been shown to form a complex with β PDGF-R when the two are overexpressed, and coexpression of E5, 16K, and β PDGF-R leads to a complex containing all three, suggesting that E5's association with β PDGF-R may involve 16K as an intermediate to which each binds [25] (see section F for critical discussion).

Functionally, 16K is thought to be involved in acidification of endosomes and thus receptor-ligand dissociation and degradation [21, 26] and perhaps gap-junctions involved in cell-cell communication [21]. HPV16 E5 does appear to inhibit acidification of endosomes [75], which could explain the increased receptor half-life and degree of receptor recycling observed for EGF-R in the presence of E5 [76]; however, the effect on receptors would be expected to be relatively nonspecific, and there is some evidence that the effect of E5 on PDGF-R levels is one of *down*regulation [54]. E5 may interfere with the normal role of 16K, as suggested by two observations: transfection of a defective 16K mutation had a phenotype similar to that resulting from E5 transfection, and cotransfection of E5 and 16K is less transforming than transfection of E5 alone, suggesting that overexpression of 16K can partially override the biological effects of E5 [2].

E5 mutations which abrogate dimerization, but not transformation, may retain binding to 16K, while mutation of Q17, which inhibits E5 transformation but not dimerization, leads to greatly reduced 16K binding [25]. However, the correlation between 16K binding and tranforming activity is only partial: several mutations in the C-terminus of E5 which are transformation defective show approximately wild-type levels of 16K binding [27], and deletion of C-terminal residues 31–44 does not eliminate 16K binding [25].

Mutational analysis of 16K indicates that the fourth transmembrane region is crucial for interaction with E5; 16K mutation E143R showed substantial reduction of E5 binding, and E5 mutations Q17E and Q17D show reduced 16K binding, but binding between the two mutated proteins 16K E143R and E5 Q17E or Q17D is greater than that between the two wild-type proteins, suggesting attraction of opposite charges at these two sites [2].

➢ See **Table I.6** for a summary of the effect of various mutations on binding to 16K.

I.H. EGF-R and EGF, and Other Growth Factor Receptors

Co-overexpression of BPV1 E5 and EGF-R, or overexpression of E5 and treatment with EGF leads to substantially greater focus formation in NIH3T3 cells than overexpression of E5 alone, overexpression of EGF-R alone, or treatment with EGF, which lead to only slight focus formation [46]. Other signs of interaction between E5 and elements of the EGF/EGF-R pathway include increased phosphorylation and half-life of EGF-R in the presence of E5 [46], and the formation of a complex between E5 and chimeric receptors containing the cytoplasmic portion of EGF-R, with concommitant phosphorylation of the receptor, focus formation and anchorage independent growth [14]. E5 has also been shown to stablize the EGF-R+EGF complex, reducing the rate of dissociation [79].

On the other hand, other studies have failed to find interactions between E5 and EGF-R. No evidence of complexes between E5 and endogenous EGF-R was observed in COS cells [25], epithelial NMuMMG (normal mouse mamary gland) cells [53], or 32D cells (a mouse myeloid line) [28]. E5 did not induce EGF-R phosphorylation in several studies [25, 28, 53]. Transfection of E5 into NMuMG cells, which lack detectable levels of endogenous β PDGF-R but express substantial amounts of EGF-R, does not lead to induction of DNA-synthesis nor tumorigenicity unless co-transfected with β BDGF-R [53]. Likewise, co-transfection of β PDGF-R, but not EGF-R, and E5 leads to growth stimulation and tumorigenic transformation of 32D cells [28].

Where interaction of EGF-R has been observed, some degree of specficity is involved, as other oncogenes (c-*fes*, c-*src*) did not cooperate with E5 [46]. However, some cooperation between E5 and other growth factor receptors (CSF-1 receptor [46] and insulin-like growth factor I receptor [51]) has been observed. The mechanism for cooperation with EGF-R does not reflect up-regulation of TGF-α, a ligand of EGF-R, emphasizing the ligand-independent character of the interaction [46].

The observed orientation of E5 in membranes, where the C-terminal hydrophilic portion is directed away from the cytoplasm, does not readily account for binding between E5 and the cytoplasmic domain of EGF-R [14]. A fusion protein consisting of GST (glutathione *S*-transferase) and the C-terminal portion of E5 binds to α-adaptin and a related, previously unknown protein, p125 [15]; since α-adaptin is known to bind to the cytoplasmic domain of other receptors, it is possible that one of these proteins serves to link E5 and the cytoplasmic domain of EGF-R. p125 appears to undergo serine-phosphorylation when complexed with E5 [15].

I.I. Immune Response and Antibody Binding

Immune response to BPV1 E5 may be protective, as inoculation with BPV1 E5-expressing vaccinia recombinants partially protected Fischer rats against subsequent tumorigenic challenge with FR 3T3 cells transformed with BPV1 [49], in contrast to results for HPV16 E5 in an analogous study [48].

Several groups have raised antibodies to BPV1 E5, E5-containing fusion proteins, or E5-peptides [7, 54]. Antibodies raised against a fusion protein containing the C-terminus bound to full length E5 protein in vivo [7], but no other epitope mapping studies are available.

I.J. Similar Cellular and Viral Proteins: E5 Homologs or Convergent Evolution?

A protein of HTLV-1, $p12^I$, shows some superficial similarity to E5 in that it binds to 16K and contains a similar highly hydrophobic central region, including a glutamine (Q) residue which could correspond to BPV1 E5 Q17 [22]. Cotransfection of $p12^I$ and E5 vectors into C127 substantially enhances focus formation over transfection of E5 alone, although $p12^I$ by itself shows no focus formation activity [22].

Sequence similarity to E5 has also been suggested for other viral and cellular genes, including various retroviral envelope glycoproteins [22], EBV LMP-1 and FeLV(a) TM [23] and Q300, murine HC1, and human PE5L [24, 36, 78]. The sequence similarities in these relatively repetitive sequences (highly hydrophobic on the amino acid level, highly G+T rich on the DNA level) make evaluation of significance difficult, and it is not clear that these proteins need to share much beyond, perhaps, membrane association.

Only for $p12^I$ has any complementation or cooperation between E5 and these genes been explored and despite the cooperation between E5 and $p12^I$ when cotransfected, further work has suggested that interaction with 16K is not due to the regions of $p12^I$ and E5 showing sequence similarity: the portion of $p12^I$ which would correspond to the hydrophobic, 16K-binding region of E5 can be deleted without loss of association with 16K, and mutations in transmembrane domain 4 (TM4) of 16K, which is crucial to association of 16K and E5, do not prevent association of $p12^I$ and 16K [40]. Furthermore, several $p12^I$ mutations which retain the ability to bind 16K are unable to potentiate E5's focus forming activity in C127 cells [40].

Similarity between E5 and Various Viral Proteins [23]

Protein	Sequence	aa
BPV1 E5	MPNLWFLLFL GLVAAMQLLL LLFLLLFF	1–30
HTLV-I $p12^I$	RPPPAPCLLLFL PFQILSGLLF LLFLPLFF	32–63
EBV LMP-1	RPPRGPPLSSSL GLALLLLLLA LLFWLYIV	14–45

Protein	Sequence	aa
BPV1 E5	FLGLVAAMQLLLLLFLLLF	9–27
HTLV-I $p12^I$	FLPFQILSGLLFLLFLPLF	42–60
EBV LMP-1	LSSSLGL-ALLLLLLALLF	21–38
FeLV(A) TM	LISSIMG-PLLILLLILLF	161–178

II. The E5 Proteins of the HPVs

The E5 ORF of mucosal HPVs, like that of BPV1, is located downstream of the E2 ORF and encodes a highly hydrophobic, membrane associated, transforming protein. However, the sequences of the HPV E5 proteins and BPV1 E5 are very different and it is impossible to know whether they are genuinely homologous. Some limited sequence similarities have been suggested between the HPV and BPV1 E5 proteins, such as potential helix-breaking prolines and a few hydrophobic residues [12], but the HPV E5s are nearly twice as long as BPV1 E5, and are likely to form three transmembrane segments [5]. The C-terminal portion of BPV1 E5, which appears to be important for biological function, does not have an identifiably similar region in the HPV E5s. Even amongst themselves, the HPV E5s are poorly conserved; a few conserved residues may seen in sequence alignments, but the alignments themselves are uncertain.

Despite this lack of conservation, several functional similarities have been observed between BPV1 E5 and the HPV E5s, specifically HPV16 E5, HPV6/HPV11 E5a, and RhPV-1 E5a. Localization to cellular membranes is shared [16]. Although disulfide bonds are apparently not involved, HPV16 E5, like BPV1 E5, may possibly form homodimers [38]. All of these proteins are capable of transforming rodent fibroblasts (leading to DNA synthesis induction, focus formation, anchorage independence, and tumorigenicity) [12, 13, 17, 43, 44, 58, 64, 76]. All HPV E5 proteins, except RhPV-1 E5a (which has not been tested), have been shown to form a complex with 16K [13, 16, 37, 77] and to bind to several growth factor receptors in at least some studies [17, 35]. BPV1 E5 and HPV16 E5 both are able to affect EGF-R processing [76, 79]. Like BPV1 E5, RhPV-1 E5a can cooperate with *ras* to transform primary rodent epithelial cells and baby rat kidney cells [58].

However, some of these properties have not been observed for the HPV E5s in other studies, including: dimerization [16, 35], complexes between HPV16 E5 and various growth factor receptors [17], transformation of fibroblasts [44], and modifications of EGF-R processing [64]. Moreover, some activities of BPV1 E5 have notably not been detected for the HPV E5s, including ligand-independent activation (phosphorylation) of EGF-R [76], cooperation with PDGF in transformation [43, 76], and binding to p125 or α-adaptin [37].

Other activities have been demonstrated for HPV E5s but either have not been tested for or observed for BPV1 E5, including: cooperation with HPV16 E7 to transform primary rodent cells [4], for which HPV16 E5 has a greater effect than HPV6 E5a [77]; cooperation with EGF to increase levels of c-fos and c-jun expression in 3T3 cells [43], which appears to correlate with increased activity of the viral enhancer [4]; increased MAP kinase activity, with or without stimulation of cells by EGF [32]; and interference with cell-cell communication via gap junctions, which correlates with dephosphorylation of connexin 43 [56].

The following sections review what is known about the HPV E5s in greater detail. As appropriate, tables summarizing mutational studies are provided at the end of the chapter.

II.A. Localization

When overexpressed in COS cells, fusion proteins consisting of HPV16 E5 or HPV6 E5a plus N-terminal epitope tags (RNGS epitope + HPV16 E5, AU1 epitope + HPV6 E5a) are localized to various membranes; the HPV16 E5 fusion protein is primarily localized to the endoplasmic reticulum (ER), but is also seen in the nuclear membrane and golgi apparatus, while the HPV6 E5a fusion protein is primarily located in the golgi apparatus, and also seen in the ER and nuclear membrane [16]. HPV11 E5a transfected into NIH3T3 and C127 cells has been reported to be localized primarily in the nucleus, although the photographs accompanying the report do not rule out localization to the nuclear membrane [12].

II.B. Mobility and Dimerization

Monomeric HPV16 E5 migrates as an approximately 10 kd band [35], and HPV6 E5a migrates as an approximately 12 kd band [12]. A 20 kd band on a reducing gel was identified with anti-HPV16 E5 antibodies in samples from HPV16-positive cervical scrapes, suggesting the presence of HPV16 E5

dimerization via a mechanism other than disulfide bonding [38]; however, overexpressed HPV16 E5 in COS cells was observed almost entirely as a monomeric (10 kd) band in another study [35]. When overexpressed in COS cells, no sign of oligomerization was seen for epitope tagged RNGS+HPV16 E5 or AU1+HPV6 E5a fusion proteins [16].

II.C. Transformation

HPV16 E5, HPV6 E5a, HPV11 E5a and RhPV-1 E5a have been shown to be transforming in a variety of assays, including anchorage independence, focus/colony formation, immortalization and tumorigenicity (see below for references). The transforming activity of the HPV E5s seems to be somewhat weaker than that of BPV1 E5, at least in the absence of stimulation with EGF [64]. In fact, one study failed to observe any of a variety of transforming activities in C127 cells and 3T3 cells transfected with HPV16 E5, including focus formation, anchorage independence, morphological transformation, growth in low serum, or tumorigenicity [44].

DNA synthesis is increased in serum-starved primary human keratinocytes transfected with HPV16 E5 or HPV6 E5a, and treatment with EGF leads to additional increases in activity [76].

Anchorage independent growth is seen in both 3T3 cells and C127 cells transfected with HPV16 E5 or HPV6 E5a; a cooperative increase in activity is observed upon addition of EGF but not PDGF [43, 76]. In one study, HPV16 E5 alone did not lead to anchorage independence of 3T3 cells, while HPV16 E5 and EGF together led to substantially greater growth than EGF alone [64]. HPV11 E5a leads to anchorage independence in 3T3 cells but only marginally in C127 cells [12]. BRK (baby rat kidney) cells show increased anchorage independence when transfected with HPV16 E5 or HPV6 E5a [4, 77]; cooperation with HPV16 E7 (but not HPV16 E6) is observed [4, 77], and c-raf is able to functionally substitute for E5 or E5a [77]. Anchorage independence of BRK cells overexpressing c-ras is increased by co-transfection with RhPV-1 E5 [58].

Focus formation, higher growth rate, and higher saturation density have been observed in 3T3 cells (but not C127 cells) transfected with HPV11 E5a [12]. Other studies have indicated that transfection with HPV16 E5 does not lead to focus formation nor affect growth rate in 3T3 cells in standard tissue culture, although higher saturation density is observed [76]. Colony formation is observed in BRK cells transfected with HPV16 E5 or HPV6 E5a [4, 77]. Colony formation of BRK cells overexpressing c-ras is increased by co-transfection with RhPV-1 E5 [58]. Cooperation of E5 with growth factors normally found in serum is indicated by the greater growth rate of HPV16 E5 transfected cells in low-serum medium compared to control cells [43], although others have not seen a growth advantage for HPV16 E5 or HPV6 E5a-positive cells in low serum [77].

Prolonged lifespan and immortalization have been observed in BRK cells co-transfected with c-ras and RhPV-1 E5 or with HPV16 E7 and HPV16 E5 [4, 58, 77].

Induction of tumors in nude mice has been seen with 3T3 cells transfected with HPV16 E5 [43]. BRK cells overexpressing c-ras alone did not lead to tumors in nude mice while cells co-transfected with RhPV-1 E5 did [58]. In a study which found little or no evidence of transformation of rodent fibroblasts (3T3 and C127) by HPV16 E5, rodent keratinocytes (p117 cells) transfected with HPV16 E5 were tumorigenic [44]; the histology of the HPV16 E5-induced tumors suggests the formation of benign papillomas in contrast to BPV1 E5 transfected cells, which led to tumors with the appearance of squamous cell carcinomas [44]. The contrast observed in this study between fibroblasts and keratinocytes suggests a possible link with the tissue tropisms of the fibropapillomaviruses (BPV1) and the true papillomaviruses (HPV16) [44], although transformation of fibroblasts by HPV16 E5, HPV6 E5a and HPV11 E5a calls this into question.

➢ See **Table II.1** for a summary of the effect of various mutations to HPV11 E5a on anchorage independent growth and binding to 16K.

II.D. 16K, Endosomal Acidification, and Gap Junctions

HPV16 E5 and HPV6 E5a both co-immunoprecipitate overexpressed 16K [16, 77]. HPV16 E5 has been shown to complex with endogenous 16K in human keratinocytes as well [37]. Controls

involving overexpression of other membrane associated proteins (IL-2R and MHCI) indicate that this association is somewhat specific [16], although HPV16 E5 has been shown to bind a wide range of cellular receptors as well as the vesicular stomatitis virus glycoprotein, suggesting complexes may involve aggregation of hydrophobic transmembrane domains [35].

HPV16 E5 inhibits acidification of endosomes in human keratinocytes, supporting hypotheses of one function of E5+16K interactions [75]. Inhibition of endosome acidification with chloroquine affects EGF-R processing in a fashion similar to the effects of E5, leading to prolonged retention of undegraded EGF-R in intracellular vesicles [75].

It has been suggested that interaction of BPV1 E5 with 16K might interfere with gap junctions, since 16K may form gap-junction-like channels, although connexins are generally thought to be the structural component of vertebrate gap junctions [21]. Cell-cell communication via gap junctions, as assessed by dye coupling, is disrupted by HPV16 E5 in HaCaT cells, a human keratinocyte line; also observed in the same system was dephosphorylation of connexin 43 (Cx43) [56]. Such a connection between inhibition of dye coupling and dephosphorylation of Cx43 has been seen in other systems [47, 59], sited in [56]. If disrupting cell-cell communication is indeed a significant function of E5, E5 may interfere with tumor-suppressor signaling as well as enhancing growth stimulation.

HPV11 E5a mutations which have been shown to be substantially defective for 16K binding have also been transformation (anchorage independence) defective; however, retention of 16K binding is not enough to assure transformation [13].

➢ See **Table II.1** for a summary of the effect of various mutations to HPV11 E5a on anchorage independent growth and binding to 16K.

II.E. PDGF and PDGF-R

Complexes between AU1-epitope-tagged HPV6 E5a and EGF-R, immature PDGF-R, and the erbB2 receptor were observed when AU1–E5a and the receptors were co-expressed in COS cells; the same study failed to find complexes between RNGS-tagged HPV16 E5 and these receptors [17]. However, in another study, unmodified HPV16 E5 was observed in complexes with PDGF-β-R, EGF-R, and CSF-1-R, as well as p185*neu* and the vesicular stomatitis virus glycoprotein [35].

3T3 cells transfected with HPV16 E5 were not sensitive to treatment with PDGF, showing no enhanced anchorage independent growth over cells not exposed to PDGF [43]. Weak cooperation between HPV16 E5 and PDGF is seen in the elevated expression levels of c-fos in 3T3 cells [43].

II.F. EGF and EGF-R

Several assays demonstrate cooperative behavior between HPV E5s and EGF. Cooperation between EGF and HPV16 E5 or HPV6 E5a leads to increase anchorage independent growth in 3T3 cells [43, 64, 76], with one study finding that transfection of E5 or E5a alone (in the absence of EGF) had no effect [64]. Induction of DNA synthesis in primary human foreskin keratinocytes transfected with E5 or E5a is increased by addition of EGF, although EGF is not necessary [76]. Phosphorylation of EGF-R is greater for the combination of EGF and HPV16 E5 than for EGF alone and, importantly, in the absence of EGF, no receptor phosphorylation was observed regardless of the presence of E5 [76]. Cooperative increases in MAP kinase activity in HT1080 (transformed human fibrosarcoma) cells [32] and colony formation and anchorage independent growth in BRK (baby rat kidney) cells [4, 77] have been shown for the combination of HPV16 E5 and EGF.

Evidence is somewhat mixed on whether HPV16 E5 interacts directly with EGF-R. In one study, an AU1 epitope-tagged HPV6 E5a fusion protein was found in complexes with EGF-R when both were overexpressed in COS cells, but RNGS epitope-tagged HPV16 E5 was not, nor was the tagged HPV16 E5 found in complexes with other growth factor receptors [17]. However, another study did find complexes between (untagged) HPV16 E5 and EGF-R as well as other receptors [35].

Neither EGF-R levels nor half-life were affected by HPV16 E5 in one study when both were overexpressed in 3T3 cells [64], but in another study measuring response of endogenous EGF-R to EGF, receptor levels, half-life, and recycling to the cell surface were all increased in primary human

foreskin keratinocytes transfected with HPV16 E5 [76]. Inhibition of acidification of endosomes by HPV16 E5 may account for part of the modified processing of EGF-R in the latter study, since treatment of cells with chloroquine, which also interferes with endosomal acidification, leads to prolonged half-life by retention of undegraded receptor in endosomes [76]; however, increased receptor recycling is not observed with chloroquine treatment, suggesting that E5's effects involve an additional mechanism.

p125, observed in complex with BPV1 E5 fusion proteins [15] and suggested as a possible intermediary in BPV1 E5+EGF-R complexes, was not identified as a co-immunoprecipitate of HPV16 E5 from cell lysate of a human keratinocyte cell line [37]. However, it should be noted that p125 was found in complex with a GST–BPV1 E5 fusion protein containing only the C-terminal protion of BPV1 E5 [15], so failing to find p125 in complex with full-length HPV16 E5 may not be overly meaningful.

II.G. c-fos and c-jun Expression and AP1 Enhancers

Levels of c-fos and c-jun mRNA may be up-regulated by binding of ligand to EGF-R or β PDGF-R. Cells transfected with HPV16 E5 show additional up-regulation of c-fos mRNA when treated with fetal calf serum, EGF, or PDGF, with response to EGF being greater than response to PDGF [4, 43]. The time course of c-fos mRNA production remains the same in response to EGF in the presence or absence of E5, with peak levels appearing about 30 min. after addition of EGF, but the levels are elevated in the presence of E5 [43]. The effect of EGF on c-jun levels is also increased by the presence of E5, although the increase is smaller than for c-fos [4].

E5 alone (i.e., without treatment of cells with growth factors) is sufficient to slightly increase c-fos and c-jun levels, possibly indicating either ligand-independent activation of growth-factor receptors or increased sensitivity to low levels of growth factors in the media [4].

The AP1 transcription factors are made up of c-fos+c-jun homo-/heterodimers, and the HPV enhancer region contains AP1 binding sites. Since E5 can induce greater expression of c-fos and c-jun, it might be expected to increase HPV enhancer activity; one study has shown that the HPV16 enhancer is indeed more active in 3T3 cells expressing E5 [4].

II.H. c-ras, c-raf and MAP Kinase Activity

Growth factors lead to cell growth at least in part through mitogenic signaling pathways which include binding and activation of the growth factor receptors, upregulation of c-ras, upregulation of c-raf, activation of the MAP kinase kinase, and activation of the MAP kinase [32]. The effect of HPV E5 proteins on these pathways has been shown at several points: at least in some studies, E5 forms complexes with EGF-R [17, 35]; the activation of EGF-R by EGF is enhanced in the presence of E5 [76]; E5-positive cells show more sensitivity to c-ras [58]; c-raf and E5 appear to have exchangeable roles in cooperating with HPV16 E7 [77]; and MAP kinase activity in response to EGF is increased and prolonged, with some E5-induced increase in activity being observed even in the absence of EGF [32].

II.I. Immune Response and Antibody Binding

Sera from patients with HPV16-positive cervical cancer and a variety of control patients showed weak IgA and IgG antibody reactivity with five synthesized 20-amino acid fragments of HPV16 E5; no fragment was more (or less) reactive than the others, and no consistent IgM reactivity was observed [18]. Despite these signs of immunologic response to HPV E5 proteins, the response generally appears to be weak. No HPV16 E5-reactive human sera were identified in a study in which the same samples had anti-L2 reactivity [39]. In addition, in contrast to BPV1 E5, inoculation with HPV16 E5-expressing vaccinia recombinants did not protect Fischer rats against subsequent tumorigenic challenge with FR 3T3 cells transformed with the autologous papillomavirus genome [48, 49].

Several groups have raised antibodies against HPV E5 proteins, or fragments thereof, and demonstrated specific binding to the corresponding E5 protein [11, 12, 35, 38]; in addition, cross-reactivity between anti-HPV11 E5 antibodies and HPV6 E5 protein has been shown [11]. One group has preferred to study biological activity of epitope-tagged E5 fusion proteins, at least in part due to difficulty in raising effective anti-E5 antibodies [16, 17].

III. Other “E5” Proteins

Several other papillomavirus open reading frames have either been labeled “E5” proteins or been suggested to have similar functions.

In BPV1, an ORF in the same region as E5 has been dubbed E5B, and associated with specific biological activity [55]. BPV1-transformed C127 cells express a set of five apparently cellular proteins which are not seen in untransformed cells; the changes include modifications of cellular membrane proteins grp100 and calreticulin. These changes appear to be dependent on an intact E5B ORF, which contains a start codon at nt 4013 and which has the potential to encode a 52 aa peptide with a hydrophobicity profile similar to BPV E5 and the E5(a) proteins of genital HPVs (but not HPV6 E5b). Neither anchorage independence nor focus formation were affected by E5B knockout mutations. The association with modification of membrane proteins led the authors to suggest that E5B may alter normal ER mediated protein processing, but little direct evidence is available.

HPV6 and HPV11 also contain a second ORF downstream of E5a which is called E5b. This E5b shows no sequence similarity to other E5 proteins and does not appear to code for a membrane-associated protein; little is known about the biological function, if any, of this ORF.

RhPV likewise contains an E5b ORF just downstream of E5 [58], which, when overexpressed, does not appear to be transforming.

BPV4 contains an ORF, E8, which occupies a position in the genome corresponding to E6 in other papillomaviruses; however, the predicted protein sequence does not show any similarity to E6. E8 is membrane-associated and may induce anchorage independent growth, suggesting that it may play a role analogous to E5 of other papillomaviruses [60].

Table I.1 Effect of Mutations to BPV E5 on Focus Formation

Mutations	Sequence	Activity (% wt) in C127 cells	[Ref.]	Activity (% wt) in NIH3T3 cells	[Ref.]
wt	MPNL....WFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF				
aa3-4 →TSRG	--TS..RG------ ---------- ---------- ---------- ----	active	[6]		
aa3-4 →TSRPRG	--TSRPRG------ ---------- ---------- ---------- ----	active	[6]		
aa3-8 →TSRMVSIV	--TS..RMVSIV-- ---------- ---------- ---------- ----	≈10	[34]		
wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF				
aa3-13 → TSRMVSIVLGI	--TSRMVSIV LGI------- ---------- ---------- ----	active	[6]		
aa3-4 →TG	--TG------ ---------- ---------- ---------- ----	active	[6]		
aa3-4 →ϕ	--..------ ---------- ---------- ---------- ----	active	[6]		
A15E,Q17K	---------- ----E-K--- ---------- ---------- ----	1	[33]		
A15T,Q17H	---------- ----T-H--- ---------- ---------- ----	112,114	[33, 42]		
A15V,L21F,L22V	---------- ----V----- FV-------- ---------- ----	96	[33]		
M16R	---------- -----R---- ---------- ---------- ----	0	[33]		
Q17C	---------- ------C--- ---------- ---------- ----			<1	[50]
Q17D	---------- ------D--- ---------- ---------- ----	200	[72]	<1,10	[50, 72]
Q17E	---------- ------E--- ---------- ---------- ----	278	[72]	>20,136	[50, 72]

Table I.1 (cont.) Effect of Mutations to BPV E5 on Focus Formation

Mutations	Sequence	Activity (% wt) in C127 cells	[Ref.]	Activity (% wt) in NIH3T3 cells	[Ref.]
wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF				
Q17E,W32C,D33$	---------- ------E--- ---------- -C.....			<1	[50]
Q17E,D33A	---------- ------E--- ---------- --A------- ----			>20	[50]
Q17E,D33C,H34$	---------- ------E--- ---------- --C.....			<1	[50]
Q17E,H34A	---------- ------E--- ---------- ---A------ ----			>20	[50]
Q17E,H34A,E36A	---------- ------E--- ---------- ---A-A---- ----			>20	[50]
Q17E,H34A,F35A	---------- ------E--- ---------- ---AA----- ----			>20	[50]
Q17E,H34C,F35$	---------- ------E--- ---------- ---C.....			>20[a]	[50]
Q17E,F35A	---------- ------E--- ---------- ----A----- ----			>20	[50]
Q17E,F35A,E36A	---------- ------E--- ---------- ----AA---- ----			>20	[50]
Q17E,F35C,E36$	---------- ------E--- ---------- ----C.....			>20	[50]
Q17E,E36A	---------- ------E--- ---------- -----a---- ----			>20	[50]
Q17E,E36C,C37$	---------- ------E--- ---------- -----C....			>20	[50]
Q17F	---------- ------F--- ---------- ---------- ----	0	[72]	0	[72]
Q17G	---------- ------G--- ---------- ---------- ----	4,1,12	[33, 72, 42]	<1,0	[50, 72]
Q17H	---------- ------H--- ---------- ---------- ----	86	[72]	1–5,2	[50, 72]
Q17K	---------- ------K--- ---------- ---------- ----	11	[72]	>20,7	[50, 72]
Q17L	---------- ------L--- ---------- ---------- ----	<5,{2,<1}	[27, 42]	<1	[50]
Q17M	---------- ------M--- ---------- ---------- ----			<1	[50]
Q17N	---------- ------N--- ---------- ---------- ----	2	[72]	<1,0	[50, 72]
Q17R	---------- ------R--- ---------- ---------- ----	2	[72]	<1,3	[50, 72]
Q17S	---------- ------S--- ---------- ---------- ----	293	[72]	39	[72]
Q17V	---------- ------V--- ---------- ---------- ----			<1	[50]
Q17Y	---------- ------Y--- ---------- ---------- ----			<1	[50]
Q17$	---------- ------....	0	[33]		

Table I.1 (cont.) Effect of Mutations to BPV E5 on Focus Formation

Mutations	Sequence	Activity (% wt) in C127 cells	[Ref.]	Activity (% wt) in NIH3T3 cells	[Ref.]
wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF				
L18R,L19K,L21F	---------- -------RK- F--------- ---------- ----	2	[33]		
L19V	---------- --------V- ---------- ---------- ----	88	[33]		
L22M	---------- ---------- -M-------- ---------- ----	78	[33]		
F23I	---------- ---------- --I------- ---------- ----	81	[33]		
F28A	---------- ---------- -------A-- ---------- ----			>20	[50]
L29A	---------- ---------- --------A- ---------- ----			>20	[50]
L29F,T40S	---------- ---------- --------F- ---------S ----	66	[33]		
V30A	---------- ---------- ---------A ---------- ----			>20	[50]
V30L,F35S	---------- ---------- ---------L ----S----- ----	6	[33]		
Y31A	---------- ---------- ---------- A--------- ----			>20	[50]
Y31F	---------- ---------- ---------- F--------- ----	54,69	[33, 34]		
Y31S	---------- ---------- ---------- S--------- ----	6,13,15	[33, 34, 71]		
W32A	---------- ---------- ---------- -A-------- ----			>20	[50]
W32C,C37A,C39A,F44C	---------- ---------- ---------- -C----A-A- ---C			<1	[50]
W32S	---------- ---------- ---------- -S-------- ----	3[a],1[b]	[33]		
D33A,E36A	---------- ---------- ---------- --A--A---- ----			<1	[50]
D33A,F35A	---------- ---------- ---------- --A-A----- ----			<1	[50]
D33A,H34A	---------- ---------- ---------- --AA------ ----			<1	[50]
D33C,C37A,C39A,P43C	---------- ---------- ---------- --C---A-A- --C-			<1	[50]
D33N	---------- ---------- ---------- --N------- ----	36	[33]		
D33V	---------- ---------- ---------- --V------- ----	3[a], 2[b]	[33]		
H34C,C37A,C39A,L42C	---------- ---------- ---------- ---C--A-A- -C--			>20[c]	[50]
H34N	---------- ---------- ---------- ---N------ ----	90	[33]		

[a] Transfection with complete BPV1 genome.
[b] Transfection with vector expressing only E5.
[c] Foci smaller than for wt.

Table I.1 (cont.) Effect of Mutations to BPV E5 on Focus Formation

Mutations	Sequence	Activity (% wt) in C127 cells	[Ref.]	Activity (% wt) in NIH3T3 cells	[Ref.]
wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF				
H34Q	---------- ---------- ---------- ---Q------ ----	72	[33]		
H34Q,E36D,G41A	---------- ---------- ---------- ---Q-D---- A---	71	[33]		
H34Y	---------- ---------- ---------- ---Y------ ----	100	[33]		
F35C,C37A,C39A,G41C	---------- ---------- ---------- ----C-A-A- C---			<1	[50]
F35I,T40I	---------- ---------- ---------- ----I----I ----	81	[33]		
E36A	---------- ---------- ---------- -----A---- ----	91	[33]		
E36C,C37A,C39A,T40C	---------- ---------- ---------- -----CA-AC ----			5–20	[50]
E36D,T40I	---------- ---------- ---------- -----D---I ----	57	[33]		
E36K	---------- ---------- ---------- -----K---- ----	59	[33]		
C37A,C39A	---------- ---------- ---------- ------A-A- ----			<1	[50]
C37A,S38C,C39A	---------- ---------- ---------- ------ACA- ----			5–20[c]	[50]
C37R	---------- ---------- ---------- ------R--- ----	5	[33]		
C37S	---------- ---------- ---------- ------S--- ----	4[d]	[33]		
C37S,C39S	---------- ---------- ---------- ------S-S- ----	1[d],1	[33, 34]		
S38N	---------- ---------- ---------- -------N-- ----	79	[33]		
C39R	---------- ---------- ---------- --------R- ----	11	[33]		
C39S	---------- ---------- ---------- --------S- ----	4	[33]		

[c] Foci smaller than for wt.

[d] Levels of E5 substantially lower than wt.

Table I.1 (cont.) Effect of Mutations to BPV E5 on Focus Formation

Mutations	Sequence	Activity (% wt) in C127 Cells	Ref.
BPV1	MPNLWFLLFL GLV.AAMQLLL LLFLLLFFLV YWDHFECSCT GLPF	100%	
HR15	---------- ---VIVLVLA- VVIVLVLA-- ---------- ----	+	[34]
HR15,Y31F	---------- ---VIVLVLA- VVIVLVLA-- F--------- ----	72% of HR15	[34]
HR15,Y31S	---------- ---VIVLVLA- VVIVLVLA-- S--------- ----	21% of HR15	[34]
HR15,C37S,C39S	---------- ---VIVLVLA- VVIVLVLA-- ------S-S- ----	1% of HR15	[34]
HR24	---------- ---VIVLVLA- VIFILLIA-- ---------- ----	–	[34]
HR4	---------- ---VIVLVLA- VFLFFFIA-- ---------- ----	–	[34]
HR6	---------- ---FVIIIFA- VFFFIILA-- ---------- ----	–	[34]
HR9	---------- ---IIFLFLA- VLIVIFIA-- ---------- ----	–	[34]
HR16	---------- ---FVIIIFA- VFLFFFIA-- ---------- ----	–	[34]
HR122	---------- ---IFLVVFA- VIIFLFLA-- ---------- ----	–	[34]
HR127	---------- ---FFFIILA- VFLFFFIA-- ---------- ----	–	[34]
HR126	---------- ---FFFIILA- VIIFLFLA-- ---------- ----	–	[34]
wt/15Q	---------- ---.------- VVIV-VLA-- ---------- ----	5–19	[42]
wt/15	---------- ---.---L--- VVIV-VLA-- ---------- ----	<5	[42]
wt/25Q	---------- ---.------- VIFI--VA-- ---------- ----	20–50	[42]
wt/25	---------- ---.---L--- VIFI--VA-- ---------- ----	<5	[42]
wt/16Q	---------- ---.------- VFLFFFIA-- ---------- ----	<5	[42]
wt/16	---------- ---.---L--- VFLFFFIA-- ---------- ----	<5	[42]
15/wtQ	---------- ---VIVL--A- ---------- ---------- ----	>50	[42]
15/wt	---------- ---VIVLV-A- ---------- ---------- ----	<5	[42]
15/15Q	---------- ---VIVL--A- VVIV-VLA-- ---------- ----	>50	[42]
15/15	---------- ---VIVLV-A- VVIV-VLA-- ---------- ----	20–50	[42]
15/25Q	---------- ---VIVL--A- VIFI--VA-- ---------- ----	>50	[42]
15/25	---------- ---VIVLV-A- VIFI--VA-- ---------- ----	<5	[42]

Table I.1 (cont.) Effect of Mutations to BPV E5 on Focus Formation

Mutations	Sequence	Activity (% wt) in C127 Cells	Ref.
BPV1	MPNLWFLLFL GLV.AAMQLLL LLFLLLFFLV YWDHFECSCT GLPF	100%	
15/16Q	---------- ---VIVL--A- VFLFFFIA-- ---------- ----	20–50	[42]
15/16	---------- ---VIVLV-A- VFLFFFIA-- ---------- ----	<5	[42]
16/wtQ	---------- ---FVII-FA- ---------- ---------- ----	>50	[42]
16/wt	---------- ---FVIIIFA- ---------- ---------- ----	<5	[42]
16/15Q	---------- ---FVII-FA- VVIV-VLA-- ---------- ----	>50	[42]
16/15	---------- ---FVIIIFA- VVIV-VLA-- ---------- ----	20–50	[42]
16/25Q	---------- ---FVII-FA- VIFI--VA-- ---------- ----	20–50	[42]
16/25	---------- ---FVIIIFA- VIFI--VA-- ---------- ----	<5	[42]
16/16Q	---------- ---FVII-FA- VFLFFFIA-- ---------- ----	<5	[42]
16/16	---------- ---FVIIIFA- VFLFFFIA-- ---------- ----	<5	[42]
9/wtQ	---------- ---IIFL--A- ---------- ---------- ----	20–50	[42]
9/wt	---------- ---IIFLF-A- ---------- ---------- ----	<5	[42]
9/15Q	---------- ---IIFL--A- VVIV-VLA-- ---------- ----	>50	[42]
9/15	---------- ---IIFLF-A- VVIV-VLA-- ---------- ----	20–50	[42]
9/25Q	---------- ---IIFL--A- VIFI--VA-- ---------- ----	>50	[42]
9/25	---------- ---IIFLF-A- VIFI--VA-- ---------- ----	<5	[42]
9/16Q	---------- ---IIFL--A- VFLFFFIA-- ---------- ----	<5	[42]
9/16	---------- ---IIFLF-A- VFLFFFIA-- ---------- ----	<5	[42]
E5/*neu*	-----V-FI- AT-.EGVL--- --VV-V-I-- ---------- ----	5–20[e,f]	[50]

[e] Foci smaller than for wt.
[f] NIH3T3 cells.

Table I.2 Effect of Mutations to BPV E5 on Morphological Transformation

Mutations	Sequence	Acute Morph. Transformation	Ref.
wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF	+++++	[71]
A15V,L21F,L22V	---------- ----V----- FV-------- ---------- ----	++++	[71]
A15T,Q17H	---------- ----T-H--- ---------- ---------- ----	+++++	[71]
M16R	---------- -----R---- ---------- ---------- ----	—	[71]
Q17G	---------- ------G--- ---------- ---------- ----	++	[71]
L18R,L19K,L21F	---------- -------RK- F--------- ---------- ----	—	[71]
L19V	---------- --------V- ---------- ---------- ----	+++++	[71]
L22M	---------- ---------- -M-------- ---------- ----	+++++	[71]
F23I	---------- ---------- --I------- ---------- ----	++++	[71]
V30L,F35S	---------- ---------- ---------L ----S----- ----	+	[71]
Y31F	---------- ---------- ---------- F--------- ----	+++++	[71]
Y31S	---------- ---------- ---------- S--------- ----	++++	[71]
W32S	---------- ---------- ---------- -S-------- ----	+++	[71]
D33N	---------- ---------- ---------- --N------- ----	++++	[71]
D33V	---------- ---------- ---------- --V------- ----	++	[71]
H34Q,E36D,G41A	---------- ---------- ---------- ---Q-D---- A---	+++++	[71]
C37S	---------- ---------- ---------- ------S--- ----	+++	[71]
C37S,C39S	---------- ---------- ---------- ------S-S- ----	+	[71]
C39S	---------- ---------- ---------- --------S- ----	+++	[71]

Table I.2(cont.) Effect of Mutations to BPV E5 on Morphological Transformation

Mutations	Sequence	Acute Morph. Transformation	Stable Morph. Transformation	Ref.
wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF	+	+	[54]
H34Q,E36D,G41A	---------- ---------- ---------- ---Q-D---- A---	+	N.D.	[54]
H34Q,E36D	---------- ---------- ---------- ---Q-D---- ----	N.D.	+	[54]
Q17L	---------- ------L--- ---------- ---------- ----	+	–	[54]
D33V	---------- ---------- ---------- --V------- ----	+	–	[54]
C37S,C39S	---------- ---------- ---------- ------S-S- ----	+	–	[54]
W32S	---------- ---------- ---------- -S-------- ----	+	–	[54]
V30L,F35S	---------- ---------- ---------L ----S----- ----	+	–	[54]
C37S	---------- ---------- ---------- ------S--- ----	+	–	[54]
C39S	---------- ---------- ---------- --------S- ----	+	–	[54]

N.D. = Not determined.

Table I.3 A Effect of Mutations to BPV E5 on Induction of DNA Synthesis

Mutations	Sequence	%-wt DNA Synthesis	Ref.
wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF	100%	
A15V,L21F,L22V	---------- ----V----- FV-------- ---------- ----	93	[71]
A15T,Q17H	---------- ----T-H--- ---------- ---------- ----	43	[71]
M16R	---------- -----R---- ---------- ---------- ----	−3	[71]
Q17G	---------- ------G--- ---------- ---------- ----	1	[71]
Q17L	---------- ------L--- ---------- ---------- ----	4	[42]
L18R,L19K,L21F	---------- -------RK- F--------- ---------- ----	1	[71]
L19V	---------- --------V- ---------- ---------- ----	75	[71]
L22M	---------- ---------- -M-------- ---------- ----	107	[71]
F23I	---------- ---------- --I------- ---------- ----	44	[71]
V30L,F35S	---------- ---------- ---------L ----S----- ----	3	[71]
Y31F	---------- ---------- ---------- F--------- ----	136	[71]
Y31S	---------- ---------- ---------- S--------- ----	21	[71]
W32S	---------- ---------- ---------- -S-------- ----	21	[71]
D33N	---------- ---------- ---------- --N------- ----	91	[71]
D33V	---------- ---------- ---------- --V------- ----	4	[71]
H34Q,E36D,G41A	---------- ---------- ---------- ---Q-D---- A---	83	[71]
C37S	---------- ---------- ---------- ------S--- ----	5	[71]
C37S,C39S	---------- ---------- ---------- ------S-S- ----	3	[71]
C39S	---------- ---------- ---------- --------S- ----	7	[71]

Mutations	Sequence	%-wt DNA Synthesis	Ref.
BPV1	MPNLWFLLFL GLV.AAMQLLL LLFLLLFFLV YWDHFECSCT GLPF	100%	
15/25	---------- ---VIVLV-A- VIFI--VA-- ---------- ----	16	[42]
HR15/25,V17Q	---------- ---VIVL--A- VIFI--VA-- ---------- ----	116	[42]
HR15W	---------- ---VIVLV-A- VVIV-VLA-- ---------- ----	74	[42]
HR15W,V17Q	---------- ---VIVL--A- VVIV-VLA-- ---------- ----	91	[42]
HR16	---------- ---FVIIIFA- VFLFFFIA-- ---------- ----	7	[42]
HR16,I17Q	---------- ---FVII-FA- VFLFFFIA-- ---------- ----	5	[42]
Control: No E5		7	[42]

Table I.3 B Effect of E5 Peptides Injected into Nucleus of Quiescent C127 Cells on Induction of DNA Synthesis

Mutations	Sequence	Concentration necessary for wt-level synthesis	Ref.
BPV1 wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF	$1\times$	
aa 35-44	....------ ----	no effect	[30]
aa 33-44	....-------- ----	no effect	[30]
aa 32-44	....--------- ----	$1000\times$	[30]
aa 31-44	.... ---------- ----	$1000\times$	[30]
aa 16-44	....----- ---------- ---------- ----	$1\times$	[30]
aa 16-34	....----- ---------- ----.....	no effect	[30]

Mutations	Sequence	%-wt Synthesis @ concentration (M)	Ref.
BPV1 wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF	100 @ 10^{-6}	[66]
C37A,C39A	---------- ---------- ---------- ------A-A- ----	≈ 0 @ 10^{-6}	[66]
27-44	....---- ---------- ----	> 50 @ 5×10^{-5}	[66]
27-44	....---- ---------- ----	> 50 @ 10^{-5}	[66]
27-44,C37A	....---- ------A--- ----	≈ 50 @ 5×10^{-5}	[66]
		≈ 50 @ 10^{-5}	[66]
27-44,C39A	....---- --------A- ----	≈ 50 @ 5×10^{-5}	[66]
		≈ 50 @ 10^{-5}	[66]
27-44,C37A,C39A	....---- ------A-A- ----	≈ 0 @ 10^{-5}	[66]
25-44	....------ ---------- ----	≈ 100 @ 6×10^{-6}	[66]
		> 50 @ 10^{-6}	[66]
25-44,C37A,C39A	....------ ------A-A- ----	≈ 0 @ 6×10^{-6}	[66]
25-44,μa	....VIFLIF ---------- ----	> 50 @ 10^{-6}	[66]
25-44,μb	....YGEVRK ---------- ----	≈ 0 @ 10^{-6}	[66]

Table I.4 Effect of Mutations to BPV E5 on Dimerization

Mutations	Sequence	Dimer Forming	Ref.
wt	MPNL....WFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF		
aa3-4 →TSRG	--TSRG..------ ---------- ---------- ---------- ----	+	[6]
aa3-4 →TSRPRG	--TSRPRG------ ---------- ---------- ---------- ----	+	[6]
wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF		
aa3-4 →ϕ	--..------ ---------- ---------- ---------- ----	+	[6]
aa3-4 →TG	--TG------ ---------- ---------- ---------- ----	+	[6]
aa3-13 → TSRMVSIVLGI	--TSRMVSIV LGI------- ---------- ---------- ----	+	[6]
A15T,Q17H	---------- ----A-H--- ---------- ---------- ----	+	[27]
A15V,L21F,L22V	---------- ----A----- FV-------- ---------- ----	+	[27]
Q17D	---------- ------D--- ---------- ---------- ----	+	[72]
Q17E	---------- ------E--- ---------- ---------- ----	+	[72]
Q17F	---------- ------F--- ---------- ---------- ----	+	[72]
Q17G	---------- ------G--- ---------- ---------- ----	+	[27, 72]
Q17H	---------- ------H--- ---------- ---------- ----	+	[72]
Q17K	---------- ------K--- ---------- ---------- ----	+	[72]
Q17L	---------- ------L--- ---------- ---------- ----	+	[27]
Q17N	---------- ------N--- ---------- ---------- ----	+	[72]
Q17R	---------- ------R--- ---------- ---------- ----	+	[72]
Q17S	---------- ------S--- ---------- ---------- ----	+	[72]
W32S	---------- ---------- ---------- -S-------- ----	+[a]	[27]
V30L,F35S	---------- ---------- ---------L ----S----- ----	+[a]	[27]
D33V	---------- ---------- ---------- --V------- ----	+	[27]
C37S	---------- ---------- ---------- ------S--- ----	+	[27]
C37A,S38C,C39A	---------- ---------- ---------- ------ACA- ----	+	[50]
C37S,C39S	---------- ---------- ---------- ------S-S- ----	−	[27]
BPV1	MPNLWFLLFL GLV.AAMQLLL LLFLLLFFLV YWDHFECSCT GLPF	mostly dimeric	[42]
Q17L	---------- ---.---L--- ---------- ---------- ----	<50% dimeric	[42]
HR15W	---------- ---VIVLV-A- VVIV-VLA-- ---------- ----	mostly monomeric	[42]
HR15W,V17Q	---------- ---VIVL--A- VVIV-VLA-- ---------- ----	mostly dimeric	[42]
HR15/25	---------- ---VIVLV-A- VIFI--VA-- ---------- ----	mostly monomeric	[42]
HR15/25,V17Q	---------- ---VIVL--A- VIFI--VA-- ---------- ----	mostly dimeric	[42]
HR16	---------- ---FVIIIFA- VFLFFFIA-- ---------- ----	mostly monomeric	[42]
HR16,I17Q	---------- ---FVII-FA- VFLFFFIA-- ---------- ----	mostly dimeric	[42]

[a] Variable gel migration suggests mixed-orientation dimers.

Table I.5.a Effect of Mutations to BPV E5 on PDGF-Receptor Binding

Mutations	Sequence	Binding Retained	Ref.
wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF	+	[54]
Q17G	---------- ------G--- ---------- ---------- ----	20–25% wt	[25]
Q17L	---------- ------L--- ---------- ---------- ----	–	[54]
V30L,F35S	---------- ---------- ---------L ----S----- ----	+	[54]
W32S	---------- ---------- ---------- -S-------- ----	+	[54]
D33V	---------- ---------- ---------- --V------- ----	–	[54]
H34Q,E36D	---------- ---------- ---------- ---Q-D---- ----	+	[54]
C37S	---------- ---------- ---------- ------S--- ----	+	[54]
C37S,C39S	---------- ---------- ---------- ------S-S- ----	–	[54]
C39S	---------- ---------- ---------- --------S- ----	+	[54]
Δ-31-44	---------- ---------- ----------	near-wt	[25]

Table I.5.b Effect of Mutations to BPV E5 on PDGF-Receptor Downregulation

Mutations	Sequence	Downregulation	Ref.
wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF	+	[54]
V30L,F35S	---------- ---------- ---------L ----S----- ----	–	[54]
W32S	---------- ---------- ---------- -S-------- ----	–	[54]
D33V	---------- ---------- ---------- --V------- ----	–	[54]
H34Q,E36D,G41A	---------- ---------- ---------- ---Q-D---- A---	+	[54]
C37S,C39S	---------- ---------- ---------- ------S-S- ----	–	[54]
C39S	---------- ---------- ---------- --------S- ----	–	[54]

Table I.5.c Effect of Mutations to BPV E5 on Phosphorylation of Immature PDGF-Receptor

Mutations	Sequence	Phosphorylates Immature PR	Ref.
wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF	+	[54]
Q17L	---------- ------L--- ---------- ---------- ----	–	[54]
V30L,F35S	---------- ---------- ---------L ----S----- ----	+	[54]
W32S	---------- ---------- ---------- -S-------- ----	+	[54]
D33V	---------- ---------- ---------- --V------- ----	–	[54]
H34Q,E36D	---------- ---------- ---------- ---Q-D---- ----	+	[54]
H34Q,E36D,G41A	---------- ---------- ---------- ---Q-D---- A---	+	[54]
C37S	---------- ---------- ---------- ------S--- ----	+	[54]
C37S,C39S	---------- ---------- ---------- ------S-S- ----	–	[54]
C39S	---------- ---------- ---------- --------S- ----	+	[54]

Table I.6 Effect of Mutations to BPV E5 on 16K Binding

Mutations	Sequence	Binding to 16K (%wt)	Ref.
wt	MPNLWFLLFL GLVAAMQLLL LLFLLLFFLV YWDHFECSCT GLPF		
A15T,Q17H	---------- ----T-H--- ---------- ---------- ----	≈ wt	[27]
A15V,L21F,L22V	---------- ----V----- FV-------- ---------- ----	≈ wt	[27]
Q17G	---------- ------G--- ---------- ---------- ----	<< wt	[29, 25, 27]
Q17L	---------- ------L--- ---------- ---------- ----	<wt	[27]
W32S	---------- ---------- ---------- -S-------- ----	>50	[27]
D33V	---------- ---------- ---------- --V------- ----	>50	[27]
C37S	---------- ---------- ---------- ------S--- ----	>50	[27]
C37S,C39S	---------- ---------- ---------- ------S-S- ----	>50	[29, 27]
H34Q,E36D,G41A	---------- ---------- ---------- ---Q-D---- A---	>50	[27]
V30L,F35S	---------- ---------- ---------L ----S----- ----	>50	[27]
BPV1	MPNLWFLLFL GLV.AAMQLLL LLFLLLFFLV YWDHFECSCT GLPF		
15W,V17Q	---------- ---VIVL--A- VVIV-VLA-- ---------- ----	<wt	[27]
15W	---------- ---VIVLV-A- VVIV-VLA-- ---------- ----	–	[27]
25W,V17Q	---------- ---VIVL-LAL VIFILLIA-- ---------- ----	≈wt	[27]
25W	---------- ---VIVLVLAL VIFILLIA-- ---------- ----	–	[27]
Δ31-44	---------- ---------- ----------	≈wt	[25]

Table II.1 Effect of Mutations to HPV11 E5 on Anchorage Independence and 16K Binding

HPV11 E5a Mutations	Anchorage Independent Growth (%wt)	16K binding (%wt)	Ref.
wt	100%	100%	
C73S	92	>100	[13]
C75S	50	50–100	[13]
C73S,C75S	41	>100	[13]
H83Q	43	>100	[13]
Q88H	47	>100	[13]
Q66E	78	>100	[13]
D39N	76	>100	[13]
P78G	36	>100	[13]
C73LTLSYCY	15	>100	[13]
aa 1–34	82	>100[a]	[13]
aa 1–45		binding observed	[16]
del. 9A,10A	4	5–49	[13]

[a] Reduced levels of E5a protein.

References

[1] A. Alderborn and S. Burnett. Regulation of DNA synthesis in division-arrested mouse C127 cells permissive for bovine papillomavirus DNA amplification. *J Virol*, 68:4349–57, 1994.

[2] T. Andresson, J. Sparkowski, D. J. Goldstein, and R. Schlegel. Vacuolar H(+)-ATPase mutants transform cells and define a binding site for the papillomavirus E5 oncoprotein. *J Biol Chem*, 270:6830–6837, 1995.

[3] S. K. Barksdale and C. C. Baker. Differentiation-specific expression from the bovine papillomavirus type 1 P2443 and late promoters. *J Virol*, 67:5605–16, 1993.

[4] V. Bouvard, G. Matlashewski, Z. M. Gu, A. Storey, and L. Banks. The human papillomavirus type 16 E5 gene cooperates with the E7 gene to stimulate proliferation of primary cells and increases viral gene expression. *Virology*, 203:73–80, 1994.

[5] V. Bubb, D. J. McCance, and R. Schlegel. DNA sequence of the HPV-16 E5 ORF and the structural conservation of its encoded protein. *Virology*, 163:243–6, 1988.

[6] A. Burkhardt, D. DiMaio, and R. Schlegel. Genetic and biochemical definition of the bovine papillomavirus E5 transforming protein. *EMBO J*, 6:2381–5, 1987.

[7] A. Burkhardt, M. Willingham, C. Gay, K. T. Jeang, and R. Schlegel. The E5 oncoprotein of bovine papillomavirus is oriented asymmetrically in Golgi and plasma membranes. *Virology*, 170:334–9, 1989.

[8] S. Burnett, N. Jareborg, and D. DiMaio. Localization of bovine papillomavirus type 1 E5 protein to transformed basal keratinocytes and permissive differentiated cells in fibropapilloma tissue. *Proc Natl Acad Sci U S A*, 89:5665–9, 1992.

[9] C. Cerni, B. Binetruy, J. T. Schiller, D. R. Lowy, G. Meneguzzi, and F. Cuzin. Successive steps in the process of immortalization identified by transfer of separate bovine papillomavirus genes into rat fibroblasts. *Proc Natl Acad Sci U S A*, 86:3266–70, 1989.

[10] E. Y. Chen, P. M. Howley, A. D. Levinson, and P. H. Seeburg. The primary structure and genetic organization of the bovine papillomavirus type 1 genome. *Nature*, 299(5883):529–34, 1982.

[11] S. L. Chen and P. Mounts. Detection by antibody probes of human papillomavirus type 6 E5 proteins in respiratory papillomata. *J Med Virol*, 29:273–83, 1989.

[12] S. L. Chen and P. Mounts. Transforming activity of E5a protein of human papillomavirus type 6 in NIH 3T3 and C127 cells. *J Virol*, 64:3226–33, 1990.

[13] S. L. Chen, Y. P. Tsao, C. M. Yang, Y. K. Lin, C. H. Huang, and S. W. Kuo. Differential induction and regulation of c-jun, junB, junD and c-fos by human papillomavirus type 11 E5a oncoprotein. *J Gen Virol*, 76:2653–2659, 1995.

[14] B. D. Cohen, D. J. Goldstein, L. Rutledge, W. C. Vass, D. R. Lowy, R. Schlegel, and J. T. Schiller. Transformation-specific interaction of the bovine papillomavirus E5 oncoprotein with the platelet-derived growth factor receptor transmembrane domain and the epidermal growth factor receptor cytoplasmic domain. *J Virol*, 67:5303–11, 1993.

[15] B. D. Cohen, D. R. Lowy, and J. T. Schiller. The conserved C-terminal domain of the bovine papillomavirus E5 oncoprotein can associate with an alpha-adaptin-like molecule: a possible link between growth factor receptors and viral transformation. *Mol Cell Biol*, 13:6462–8, 1993.

[16] M. Conrad, V. J. Bubb, and R. Schlegel. The human papillomavirus type 6 and 16 E5 proteins are membrane- associated proteins which associate with the 16-kilodalton pore-forming protein. *J Virol*, 67:6170–8, 1993.

[17] M. Conrad, D. Goldstein, T. Andresson, and R. Schlegel. The E5 protein of HPV-6, but not HPV-16, associates efficiently with cellular growth factor receptors. *Virology*, 200:796–800, 1994.

[18] J. Dillner. Mapping of linear epitopes of human papillomavirus type 16: the E1, E2, E4, E5, E6 and E7 open reading frames. *Int J Cancer*, 46:703–11, 1990.

[19] D. DiMaio, D. Guralski, and J. T. Schiller. Translation of open reading frame E5 of bovine papillomavirus is required for its transforming activity. *Proc Natl Acad Sci U S A*, 83:1797–801, 1986.

[20] D. A. Drummond-Barbosa, R. R. Vaillancourt, A. Kazlauskas, and D. DiMaio. Ligand-independent activation of the platelet-derived growth factor beta receptor: requirements for bovine papillomavirus E5-induced mitogenic signaling. *Mol Cell Biol*, 15:2570–2581, 1995.

[21] M.E. Finbow, J.D. Pitts, D.J. Goldstein, R. Schlegel, and J.B. Findlay. The E5 oncoprotein target: a 16-kDa channel-forming protein with diverse functions. *Molecular Carcinogenesis*, 4(6):441–444, 1991.

[22] G. Franchini, J. C. Mulloy, I. J. Koralnik, Monico Lo A., J. J. Sparkowski, T. Andresson, D. J. Goldstein, and R. Schlegel. The human T-cell leukemia/lymphotropic virus type I p12I protein cooperates with the E5 oncoprotein of bovine papillomavirus in cell transformation and binds the 16-kilodalton subunit of the vacuolar H+ ATPase. *J Virol*, 67:7701–4, 1993.

[23] R. F. Garry. Sequence similarities between latent membrane protein LMP-1 of Epstein- Barr virus, integral membrane protein p12I of human T cell leukemia/lymphotropic virus type 1, E5 transformation protein of bovine papillomavirus, and the transmembrane proteins of slowly transforming retroviruses (letter). *AIDS Res Hum Retroviruses*, 11:431–432, 1995.

[24] C. Geisen, H. Delius, P. Lichter, and T. Kahn. A transcribed human sequence related to the mouse HC1 and the human papillomavirus type 18 E5 genes is located at chromosome 7p13-14. *Hum Mol Genet*, 4:1337–1345, 1995.

[25] D. J. Goldstein, T. Andresson, J. J. Sparkowski, and R. Schlegel. The BPV-1 E5 protein, the 16 kDa membrane pore-forming protein and the PDGF receptor exist in a complex that is dependent on hydrophobic transmembrane interactions. *EMBO J*, 11:4851–9, 1992.

[26] D. J. Goldstein, M. E. Finbow, T. Andresson, P. McLean, K. Smith, V. Bubb, and R. Schlegel. Bovine papillomavirus E5 oncoprotein binds to the 16K component of vacuolar H(+)-ATPases. *Nature*, 352:347–9, 1991.

[27] D. J. Goldstein, R. Kulke, D. Dimaio, and R. Schlegel. A glutamine residue in the membrane-associating domain of the bovine papillomavirus type 1 E5 oncoprotein mediates its binding to a transmembrane component of the vacuolar H(+)-ATPase. *J Virol*, 66:405–13, 1992.

[28] D. J. Goldstein, W. Li, L. M. Wang, M. A. Heidaran, S. Aaronson, R. Shinn, R. Schlegel, and J. H. Pierce. The bovine papillomavirus type 1 E5 transforming protein specifically binds and activates the beta-type receptor for the platelet-derived growth factor but not other related tyrosine kinase-containing receptors to induce cellular transformation. *J Virol*, 68:4432–41, 1994.

[29] D. J. Goldstein and R. Schlegel. The E5 oncoprotein of bovine papillomavirus binds to a 16 kd cellular protein. *EMBO J*, 9:137–45, 1990.

[30] M. Green and P. M. Loewenstein. Demonstration that a chemically synthesized BPV1 oncoprotein and its C- terminal domain function to induce cellular DNA synthesis. *Cell*, 51:795–802, 1987.

[31] D. E. Groff and W. D. Lancaster. Genetic analysis of the 3′ early region transformation and replication functions of bovine papillomavirus type 1. *Virology*, 150:221–30, 1986.

[32] Z. Gu and G. Matlashewski. Effect of human papillomavirus type 16 oncogenes on MAP kinase activity. *J Virol*, 69:8051–8056, 1995.

[33] B. H. Horwitz, A. L. Burkhardt, R. Schlegel, and D. DiMaio. 44-amino-acid E5 transforming protein of bovine papillomavirus requires a hydrophobic core and specific carboxyl-terminal amino acids. *Mol Cell Biol*, 8:4071–8, 1988.

[34] B. H. Horwitz, D. L. Weinstat, and D. DiMaio. Transforming activity of a 16-amino-acid segment of the bovine papillomavirus E5 protein linked to random sequences of hydrophobic amino acids. *J Virol*, 63:4515–9, 1989.

[35] E. S. Hwang, T. Nottoli, and D. Dimaio. The HPV16 E5 protein: expression, detection, and stable complex formation with transmembrane proteins in COS cells. *Virology*, 211:227–233, 1995.

[36] T. Kahn, H. Friesl, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, L. Gissmann, J. Kramer, and Hausen zur H. Molecular cloning, analysis, and chromosomal localization of a mouse genomic sequence related to the human papillomavirus type 18 E5 region. *Mol Carcinog*, 6:88–99, 1992.

[37] B. Kell, R. J. Jewers, J. Cason, and J. M. Best. Cellular proteins associated with the E5 oncoprotein of human papillomavirus type 16. *Biochem Soc Trans*, 22:333S, 1994.

[38] B. Kell, R. J. Jewers, J. Cason, F. Pakarian, J. N. Kaye, and J. M. Best. Detection of E5 oncoprotein in human papillomavirus type 16-positive cervical scrapes using antibodies raised to synthetic peptides. *J. Gen. Virol.*, 75:2451–6, 1994.

[39] H. G. Kochel, K. Sievert, M. Monazahian, A. Mittelstadt-Deterding, A. Teichmann, and R. Thomssen. Antibodies to human papillomavirus type-16 in human sera as revealed by the use of prokaryotically expressed viral gene products. *Virology*, 182:644–54, 1991.

[40] I. J. Koralnik, J. C. Mulloy, T. Andresson, J. Fullen, and G. Franchini. Mapping of the intermolecular association of human T cell leukaemia/lymphotropic virus type I p12I and the vacuolar H+-ATPase 16 kDa subunit protein. *J Gen Virol*, 76:1909–1916, 1995.

[41] R. Kulke and D. DiMaio. Biological properties of the deer papillomavirus E5 gene in mouse C127 cells: growth transformation, induction of DNA synthesis, and activation of the platelet-derived growth factor receptor. *J Virol*, 65:4943–9, 1991.

[42] R. Kulke, B. H. Horwitz, T. Zibello, and D. DiMaio. The central hydrophobic domain of the bovine papillomavirus E5 transforming protein can be functionally replaced by many hydrophobic amino acid sequences containing a glutamine. *J Virol*, 66:505–11, 1992.

[43] P. Leechanachai, L. Banks, F. Moreau, and G. Matlashewski. The E5 gene from human papillomavirus type 16 is an oncogene which enhances growth factor-mediated signal transduction to the nucleus. *Oncogene*, 7:19–25, 1992.

[44] C. Leptak, y Ramon Cajal, R. Kulke, B. H. Horwitz, D. J. Riese 2d, G. P. Dotto, and D. DiMaio. Tumorigenic transformation of murine keratinocytes by the E5 genes of bovine papillomavirus type 1 and human papillomavirus type 16 (published erratum appears in J Virol 1992 Mar;66(3):1833). *J Virol*, 65:7078–83, 1991.

[45] D. W. Maher, L. M. Strawn, and D. J. Donoghue. Alanine mutagenesis of conserved residues in the platelet-derived growth factor family: identification of residues necessary for dimerization and transformation. *Oncogene*, 8:533–41, 1993.

[46] P. Martin, W. C. Vass, J. T. Schiller, D. R. Lowy, and T. J. Velu. The bovine papillomavirus E5 transforming protein can stimulate the transforming activity of EGF and CSF-1 receptors. *Cell*, 59:21–32, 1989.

[47] D. F. Matesic, H. L. Rupp, W. J. Bonney, R. J. Ruch, and J. E. Trosko. Changes in gap-junction permeability, phosphorylation, and number mediated by phorbol ester and non-phorbol-ester tumor promoters in rat liver epithelial cells. *Mol Carcinog*, 10:226–236, 1994.

[48] G. Meneguzzi, C. Cerni, M. P. Kieny, and R. Lathe. Immunization against human papillomavirus type 16 tumor cells with recombinant vaccinia viruses expressing E6 and E7. *Virology*, 181:62–9, 1991.

[49] G. Meneguzzi, M. P. Kieny, J. P. Lecocq, P. Chambon, F. Cuzin, and R. Lathe. Vaccinia recombinants expressing early bovine papilloma virus (bpv1) proteins: retardation of BPV1 tumour development. *Vaccine*, 8(3):199–204, 1990.

[50] A. N. Meyer, Y. F. Xu, M. K. Webster, A. E. Smith, and D. J. Donoghue. Cellular transformation by a transmembrane peptide: structural requirements for the bovine papillomavirus E5 oncoprotein. *Proc Natl Acad Sci U S A*, 91:4634–8, 1994.

[51] A. Morrione, T. DeAngelis, and R. Baserga. Failure of the bovine papillomavirus to transform mouse embryo fibroblasts with a targeted disruption of the insulin-like growth factor I receptor genes. *J Virol*, 69:5300–5303, 1995.

[52] K. Neary and D. DiMaio. Open reading frames E6 and E7 of bovine papillomavirus type 1 are both required for full transformation of mouse C127 cells. *J Virol*, 63:259–66, 1989.

[53] L. A. Nilson and D. DiMaio. Platelet-derived growth factor receptor can mediate tumorigenic transformation by the bovine papillomavirus E5 protein. *Mol Cell Biol*, 13:4137–45, 1993.

[54] L. A. Nilson, R. L. Gottlieb, G. W. Polack, and D. DiMaio. Mutational analysis of the interaction between the bovine papillomavirus E5 transforming protein and the endogenous beta receptor for platelet-derived growth factor in mouse C127 cells. *J Virol*, 69:5869–5874, 1995.

[55] M. K. O'Banion, V. D. Winn, J. Settleman, and D. A. Young. Genetic definition of a new bovine papillomavirus type 1 open reading frame, E5B, that encodes a hydrophobic protein involved in altering host-cell protein processing. *J Virol*, 67:3427–34, 1993.

[56] I. Oelze, J. Kartenbeck, K. Crusius, and A. Alonso. Human papillomavirus type 16 E5 protein affects cell-cell communication in an epithelial cell line. *J Virol*, 69:4489–4494, 1995.

[57] A. Ostman, M. Andersson, U. Hellman, and C. H. Heldin. Identification of three amino acids in the platelet-derived growth factor (PDGF) B-chain that are important for binding to the PDGF beta- receptor. *J Biol Chem*, 266:10073–7, 1991.

[58] R. S. Ostrow, Z. Liu, J. F. Schneider, R. C. McGlennen, K. Forslund, and A. J. Faras. The products of the E5, E6, or E7 open reading frames of RhPV 1 can individually transform NIH 3T3 cells or in cotransfections with activated ras can transform primary rodent epithelial cells. *Virology*, 196:861–7, 1993.

[59] M. Oyamada, H. Kimura, Y. Oyamada, A. Miyamoto, H. Ohshika, and M. Mori. The expression, phosphorylation, and localization of connexin 43 and gap-junctional intercellular communication during the establishment of a synchronized contraction of cultured neonatal rat cardiac myocytes. *Exp Cell Res*, 212:351–8, 1994.

[60] W. D. Pennie, G. J. Grindlay, M. Cairney, and M. S. Campo. Analysis of the transforming functions of bovine papillomavirus type 4. *Virology*, 193(2):614–20, 1993.

[61] L. Petti and D. DiMaio. Stable association between the bovine papillomavirus E5 transforming protein and activated platelet-derived growth factor receptor in transformed mouse cells. *Proc Natl Acad Sci U S A*, 89:6736–40, 1992.

[62] L. Petti and D. DiMaio. Specific interaction between the bovine papillomavirus E5 transforming protein and the beta receptor for platelet-derived growth factor in stably transformed and acutely transfected cells. *J Virol*, 68:3582–92, 1994.

[63] L. Petti, L. A. Nilson, and D. DiMaio. Activation of the platelet-derived growth factor receptor by the bovine papillomavirus E5 transforming protein. *EMBO J*, 10:845–55, 1991.

[64] D. Pim, M. Collins, and L. Banks. Human papillomavirus type 16 E5 gene stimulates the transforming activity of the epidermal growth factor receptor. *Oncogene*, 7:27–32, 1992.

[65] M. S. Rabson, C. Yee, Y. C. Yang, and P. M. Howley. Bovine papillomavirus type 1 3′ early region transformation and plasmid maintenance functions. *J Virol*, 60:626–34, 1986.

[66] J. A. Rawls, P. M. Loewenstein, and M. Green. Mutational analysis of bovine papillomavirus type 1 E5 peptide domains involved in induction of cellular DNA synthesis. *J Virol*, 63:4962–4, 1989.

[67] D. J. Riese 2nd and D. DiMaio. An intact PDGF signaling pathway is required for efficient growth transformation of mouse C127 cells by the bovine papillomavirus E5 protein. *Oncogene*, 10:1431–1439, 1995.

[68] J. T. Schiller, W. C. Vass, K. H. Vousden, and D. R. Lowy. E5 open reading frame of bovine papillomavirus type 1 encodes a transforming gene. *J Virol*, 57:1–6, 1986.

[69] R. Schlegel, M. Wade-Glass, M. S. Rabson, and Y. C. Yang. The E5 transforming gene of bovine papillomavirus encodes a small, hydrophobic polypeptide. *Science*, 233:464–7, 1986.

[70] J. Settleman and D. DiMaio. Efficient transactivation and morphologic transformation by bovine papillomavirus genes expressed from a bovine papillomavirus/simian virus 40 recombinant virus. *Proc Natl Acad Sci U S A*, 85:9007–11, 1988.

[71] J. Settleman, A. Fazeli, J. Malicki, B. H. Horwitz, and D. DiMaio. Genetic evidence that acute morphologic transformation, induction of cellular DNA synthesis, and focus formation are mediated by a single activity of the bovine papillomavirus E5 protein. *Mol Cell Biol*, 9:5563–72, 1989.

[72] J. Sparkowski, J. Anders, and R. Schlegel. Mutation of the bovine papillomavirus E5 oncoprotein at amino acid 17 generates both high- and low-transforming variants. *J Virol*, 68:6120–6123, 1994.

[73] J. Sparkowski, J. Anders, and R. Schlegel. E5 oncoprotein retained in the endoplasmic reticulum/cis Golgi still induces PDGF receptor autophosphorylation but does not transform cells. *EMBO J*, 14:3055–3063, 1995.

[74] A. Staebler, J. H. Pierce, S. Brazinski, M. A. Heidaran, W. Li, R. Schlegel, and D. J. Goldstein. Mutational analysis of the beta-type platelet-derived growth factor receptor defines the site of interaction with the bovine papillomavirus type 1 E5 transforming protein. *J Virol*, 69:6507–6517, 1995.

[75] S. W. Straight, B. Herman, and D. J. McCance. The E5 oncoprotein of human papillomavirus type 16 inhibits the acidification of endosomes in human keratinocytes. *J Virol*, 69:3185–3192, 1995.

[76] S. W. Straight, P. M. Hinkle, R. J. Jewers, and D. J. McCance. The E5 oncoprotein of human papillomavirus type 16 transforms fibroblasts and effects the downregulation of the epidermal growth factor receptor in keratinocytes. *J Virol*, 67:4521–32, 1993.

[77] G. F. Valle and L. Banks. The human papillomavirus (HPV)-6 and HPV-16 E5 proteins co-operate with HPV-16 E7 in the transformation of primary rodent cells. *J. Gen. Virol.*, 76:1239–45, 1995.

[78] S. Wagner, G. Cullmann, and R. Knippers. The Q300 gene: a novel transcription unit induced in simian virus 40- infected and -transformed mouse cells. *J Virol*, 65:3259–67, 1991.

[79] C. M. Waters, K.A. Overholser, A. Sorkin, and G. Carpenter. Analysis of the influences of the E5 transforming protein on kinetic parameters of epidermal growth factor binding and metabolism. *J Cell Phys*, 152(2):253–63, 1992.

[80] B. M. Willumsen, W. C. Vass, T. J. Velu, A. G. Papageorge, J. T. Schiller, and D. R. Lowy. The bovine papillomavirus E5 oncogene can cooperate with ras: identification of p21 amino acids critical for transformation by c-rasH but not v-rasH. *Mol Cell Biol*, 11:6026–33, 1991.