



ELSEVIER

Cytokine & Growth Factor Reviews 11 (2000) 283–293

Cytokine  
Growth Factor

Reviews

www.elsevier.com/locate/cytogfr

### Mini Review

# The platelet-derived growth factor $\beta$ receptor as a target of the bovine papillomavirus E5 protein

Daniel DiMaio\*, Char-Chang Lai, Dawn Mattoon

Department of Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA

## Abstract

The 44-amino acid E5 protein of bovine papillomavirus is a homo-dimeric, transmembrane protein that transforms cells by activating the platelet-derived growth factor  $\beta$  receptor in a ligand-independent fashion. The E5 protein induces receptor activation by forming a stable complex with the receptor, thereby inducing receptor dimerization, *trans*-phosphorylation of tyrosine residues in the cytoplasmic domain of the receptor, and recruitment of cellular SH2 domain-containing proteins into a signal transduction complex. Direct interactions between specific transmembrane and juxtamembrane amino acids in the E5 protein and the PDGF  $\beta$  receptor appear to drive complex formation and dimerization of the receptor. Further analysis of this unique mechanism of viral transformation promises to yield new insight into the regulation of growth factor receptor activity and cellular signal transduction pathways. © 2000 Elsevier Science Ltd. All rights reserved.

Tumor viruses are implicated in up to 15% of all human cancers [1]. In addition to their obvious medical importance, tumor viruses have also played a central role in advancing our understanding of numerous cellular processes. Studies of the mechanism of cell transformation by viruses have yielded important insights into the nature of cellular oncogenes and tumor suppressor genes, cell cycle control, signal transduction, and gene regulation. Many DNA tumor viruses have adopted a common strategy of transformation that involves the neutralization of the cellular tumor suppressor proteins p53 and p105<sup>Rb</sup> and related proteins. More recently, it has become apparent that diverse tumor viruses transform cells by encoding intrinsic viral proteins that activate cellular growth stimulatory pathways, a mechanism we have named “virocrine transformation” [2,3]. Viral transforming proteins that use this strategy include polyomavirus middle T antigen, which mimics an activated receptor tyrosine kinase, Epstein-Barr virus LMP1, which

mimics an activated tumor necrosis factor receptor, Friend leukemia virus gp55, which activates the erythropoietin receptor, and the bovine papillomavirus (BPV) E5 protein. The BPV E5 protein transforms cells by binding to and activating the platelet-derived growth factor (PDGF)  $\beta$  receptor.

Bovine papillomavirus type 1 induces the formation of fibropapillomas, benign tumors which contain a prominent proliferative dermal fibroblast component. BPV can also induce stable tumorigenic transformation of established lines of fibroblasts growing in culture. BPV-induced fibroblast transformation is due to the E5 protein, a 44-amino acid type II transmembrane protein with a very hydrophobic central domain (Fig. 1) [4–9]. In transformed cells, the E5 protein is localized largely to the membranes of the endoplasmic reticulum and Golgi apparatus [10,11], and it exists as a dimer of two identical subunits linked by disulfide bonds involving cysteine residues in the carboxyl-terminal third of the protein [4,12]. Mutational analysis revealed that much of the hydrophobic domain of the E5 protein could be substituted with other hydrophobic amino acids, so long as glutamine 17, the sole hydrophilic amino acid in that part of the protein, was

\* Corresponding author. Tel.: +1-203-785-2684; fax: +1-203-785-7023.

E-mail address: daniel.dimaio@yale.edu (D. DiMaio).

MET-PRO-ASN-LEU-TRP-PHE-LEU-LEU-PHE-LEU-GLY-LEU-VAL-ALA-ALA  
 17  
 MET-**GLN**-LEU-LEU-LEU-LEU-LEU-PHE-LEU-LEU-LEU-PHE-PHE-LEU-VAL  
 33 37 39  
 TYR-TRP-**ASP**-HIS-PHE-GLU-**CYS**-SER-**CYS**-THR-GLY-LEU-PRO-PHE

Fig. 1. Amino acid sequence of the BPV E5 protein. Amino acids critical for PDGF  $\beta$  receptor binding and activation and for cell transformation are shown in bold and numbered.

retained [12–14]. Numerous mutations were also tolerated in the more hydrophilic portions of the E5 protein [12,15]. The small size of the E5 protein and its ability to tolerate mutations suggested that it did not have intrinsic enzymatic activity but rather that it induced transformation by binding to and modulating the activity of cellular membrane proteins that regulate cell growth. Growth factor receptors were identified as potential targets of the E5 protein by two findings. First, the E5 gene displayed increased focus forming activity in NIH 3T3 cells when it was co-transfected with genes encoding receptor tyrosine kinases (RTKs) [16]. Second, acute expression of the E5 protein, like treatment with serum or purified growth factors, induced quiescent fibroblasts to commence DNA synthesis [17].

### 1. Activation of the PDGF $\beta$ receptor by the bovine papillomavirus E5 protein

The initial evidence that the E5 protein activated the PDGF  $\beta$  receptor was the demonstration that the endogenous PDGF  $\beta$  receptor was constitutively phosphorylated on tyrosine in rodent fibroblasts stably transformed by the viral protein [18]. Tyrosine phosphorylation of the PDGF  $\beta$  receptor was a rapid and dose-dependent response to transient E5 expression [18]. The E5 protein also induced constitutive tyrosine phosphorylation of endogenous PDGF  $\beta$  receptor in bovine conjunctival fibroblasts and primary human dermal fibroblasts, as well as of exogenous PDGF  $\beta$  receptor in a number of different cell types (Fig. 2) ([19–24]; L. Petti & A. Ray, Albany Medical College). In addition, the highly related E5 protein from the deer fibropapillomavirus also transformed mouse cells and activated the PDGF  $\beta$  receptor [25]. However, the BPV E5 protein did not activate other endogenous receptors, including the closely related PDGF  $\alpha$  receptor or the EGF receptor, in bovine fibroblasts [20]. In several cell types transformed by the E5 protein, both the mature cell surface form as well as an intracellular, incompletely glycosylated precursor form of the PDGF  $\beta$  receptor were tyrosine phosphorylated, and a substantial fraction of the receptor with mature carbohydrates was resistant to cell surface trypsinization

and hence was also likely to be intracellular [18]. In stably transformed cells, the E5 protein induced tyrosine phosphorylation of only a small fraction of the total cellular PDGF  $\beta$  receptor [26,27], presumably because most of the receptor was at the cell surface while most of the E5 protein was intracellular.

Several additional criteria indicated that the E5 protein activated the PDGF  $\beta$  receptor. As described below, the E5 protein formed a stable complex with the PDGF  $\beta$  receptor [21], and E5 expression induced constitutive dimerization and *trans*-phosphorylation of the PDGF  $\beta$  receptor [26] and stimulated its *in vitro* tyrosine kinase activity [18]. In E5 transformed cells, there was constitutive association between the receptor and phosphoinositol 3'kinase (PI3'kinase), phospholipase C $\gamma$  (PLC $\gamma$ ), and rasGTPase activating protein (GAP), SH2 domain-containing cellular substrates that play essential roles in the response to PDGF [23,27]. When the E5 protein was transiently expressed at a high level in fibroblasts, PDGF  $\beta$  receptor was down-regulated, as is the case for ligand-stimulated PDGF  $\beta$  receptor [28]. Finally, as is the case with PDGF treatment, co-expression of the E5 protein and the PDGF

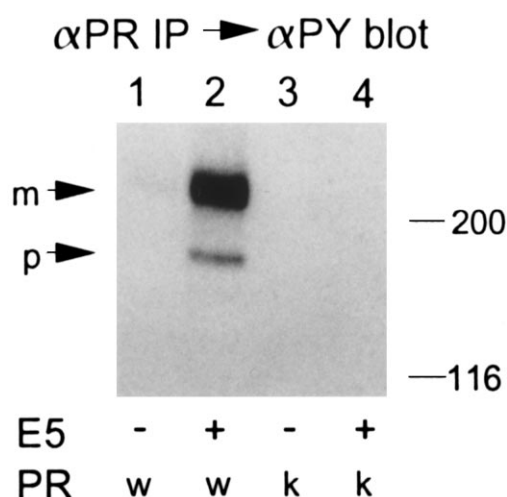


Fig. 2. Activation of the PDGF  $\beta$  receptor by the E5 protein. Ba/F3 cells expressing the wild-type (w) or kinase-negative (k) human PDGF  $\beta$  receptor were selected following infection with a control retrovirus (–) or a retrovirus expressing the BPV E5 protein (+). Activated PDGF  $\beta$  receptor was detected by immunoblotting with an antibody recognizing phosphotyrosine. Modified from Ref. [23].

$\beta$  receptor provided an anti-apoptotic signal to growth factor-deprived hematopoietic cells [22,23].

## 2. Role of PDGF $\beta$ receptor activation in E5 transformation

Several experimental approaches have been used to assess whether activation of the PDGF  $\beta$  receptor was required for cell transformation by the E5 protein. First, gene transfer experiments have been carried out in a number of cell lines lacking endogenous PDGF  $\beta$  receptor [19,22,23]. These cells were normally not susceptible to transformation by the E5 protein or by expression of the human or murine PDGF  $\beta$  receptor. However, co-expression of the E5 protein and the PDGF  $\beta$  receptor resulted in receptor activation and tumorigenic transformation or growth factor-indepen-

dent proliferation. Other receptor tyrosine kinases (RTKs) tested were not able to allow E5 transformation [22], providing further evidence that the E5 protein specifically affected the function of the PDGF  $\beta$  receptor. E5-induced growth factor-independent proliferation in these cells required the expression of a catalytically active PDGF  $\beta$  receptor tyrosine kinase [23], indicating that the PDGF  $\beta$  receptor had to activate the receptor signaling cascade to deliver a proliferative signal.

Studies have also been carried out with AG1295, a specific inhibitor of the PDGF  $\beta$  receptor tyrosine kinase [29,30]. Treatment of E5-transformed C127 mouse cells with AG1295 led to the loss of constitutive tyrosine phosphorylation of the PDGF  $\beta$  receptor and to the reversal of the morphologically transformed phenotype (Fig. 3). Removal of the inhibitor resulted in the rapid reacquisition of the transformed morphology. AG1295 also prevented growth factor-independent proliferation of Ba/F3 cells co-expressing the E5 protein and the wild-type PDGF  $\beta$  receptor [30]. In addition, C127 cell variants resistant to PDGF-mediated mitogenesis were also resistant to transformation by the E5 protein [31]. Finally, as summarized below, we observed an excellent correlation between the ability of various E5 mutants to bind to and activate the PDGF  $\beta$  receptor and to transform cells [30,32]. Taken together, these experiments provided compelling evidence that activation of the PDGF  $\beta$  receptor plays a central role in E5-induced transformation. This is similar to the situation with the product of the viral oncogene *v-sis*, a homologue of PDGF, which transforms cells by activating the PDGF receptor [33,34].

It has been reported that certain E5 mutants transformed cells without binding to or activating the PDGF  $\beta$  receptor [35,36]. Furthermore, PI3'kinase activity was elevated in cells transformed by these mutants, and the PDGF receptor kinase inhibitor did not inhibit this activity [37]. On the basis of these results, Schlegel et al. concluded that these mutants utilized alternative, PDGF  $\beta$  receptor-independent mechanisms to transform cells [35,36]. However, when we analyzed the same E5 mutants and cell lines, these mutants induced clearly increased tyrosine phosphorylation of the PDGF  $\beta$  receptor and formed stable complexes with the receptor (unpublished results). In addition, the transformed morphology of NIH3T3 cells expressing these mutants was reversed by the PDGF receptor tyrosine kinase inhibitor. It is likely that at least some of these discrepancies are the consequence of subtle but apparently important methodological differences. It remains to be seen whether the E5 protein has transforming activity that does not involve PDGF  $\beta$  receptor activation.

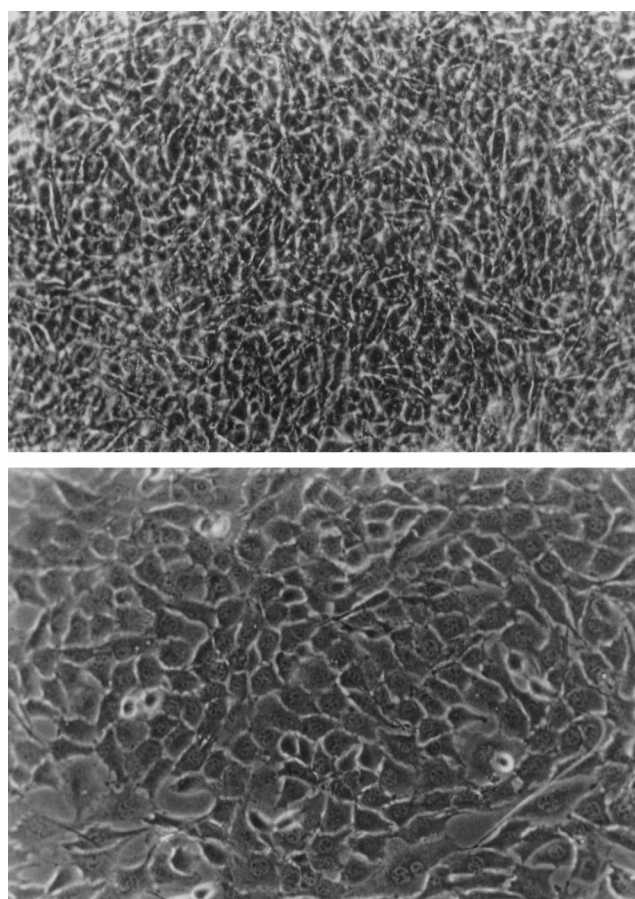


Fig. 3. Reversion of E5-transformed cells by inhibition of PDGF  $\beta$  receptor. C127 cells stably transformed by the E5 protein were treated with AG1295, a specific inhibitor of PDGF receptor tyrosine kinase (bottom panel) or left untreated (top panel). Contrast the piled-up and disorganized appearance of the untreated cells with the flat monolayer of the treated cells, characteristic of parental untransformed C127 cells.

### 3. Mechanism of PDGF $\beta$ receptor activation by the E5 protein

The study of E5-induced activation of the PDGF  $\beta$  receptor is likely to shed new light on the mechanism of receptor activation in general. Co-immunoprecipitation experiments utilizing detergent extracts of transformed C127 cells showed that the E5 protein and both mature and precursor forms of the endogenous activated PDGF  $\beta$  receptor were in a stable complex in these cells [20,21]. Phosphotyrosine blotting and velocity sedimentation indicated that the PDGF  $\beta$  receptor in the complex with the E5 protein was activated, and no other activated RTKs were detectable in this complex [21,27]. These results suggested that the E5 protein induced activation of the PDGF  $\beta$  receptor by binding to the receptor. Complex formation between the E5 protein and endogenous and foreign PDGF  $\beta$  receptors has since been confirmed in various cell systems [19,22–24,38]. In stably transformed cells, the E5 protein formed a complex with the PDGF  $\beta$  receptor only, whereas it associated with a variety of receptor tyrosine kinases, including the EGF receptor, when they were overexpressed [20].

PDGF initiates PDGF  $\beta$  receptor activation by binding to the extracellular domain of the receptor. The analysis of various mutant and chimeric PDGF  $\beta$  receptors indicated that the transmembrane and juxtamembrane domains of the PDGF  $\beta$  receptor played a major role in mediating the interaction with the E5 protein [23,24,38–40]. In fact, the E5 protein was able to complex with and activate receptors lacking the extracellular ligand binding domain, leading to the generation of an E5-induced proliferative signal [23,39]. Thus, E5-induced activation of the PDGF  $\beta$  receptor and cell transformation did not depend upon the ability of the PDGF  $\beta$  receptor to bind to its ligand, indicating that receptor activation in this system was ligand-independent.

Binding of PDGF to the PDGF  $\beta$  receptor induces receptor dimerization and *trans*-autophosphorylation of multiple tyrosine residues in the cytoplasmic domains of the two receptor subunits in the dimer [41]. To determine whether the E5 protein induced similar events, we engineered cells to co-express a full-length PDGF  $\beta$  receptor and a truncation mutant lacking almost all of the ligand binding domain. An antibody specific for the full-length receptor co-immunoprecipitated the truncated receptor when the two receptor species were co-expressed with the wild-type E5 protein, but no co-immunoprecipitation occurred in the absence of the E5 protein or in the presence of a mutant E5 protein unable to bind the PDGF  $\beta$  receptor [26]. This result indicated that the E5 protein induced oligomerization of the PDGF  $\beta$  receptor, and suggested that complex formation between the E5 pro-

tein and the PDGF  $\beta$  receptor was required for receptor oligomerization. In these experiments, the full-length receptor carried a mutation in the catalytic domain that abolished kinase activity. Nevertheless, the kinase-negative mutant was tyrosine phosphorylated when co-expressed with the wild-type E5 protein and the kinase-active receptor, indicating that the E5 protein induced the kinase-active PDGF  $\beta$  receptor to catalyze intermolecular *trans*-phosphorylation within the receptor oligomer [26]. Interestingly, the kinase-active receptor in complex with the kinase-negative form was not itself tyrosine phosphorylated, indicating that in the heteromeric complex, the kinase-active receptor cannot catalyze intramolecular tyrosine phosphorylation. This result suggested that the E5 protein induced the formation of PDGF  $\beta$  receptor dimers but not higher order oligomers, because *trans*-phosphorylation between multiple kinase-active receptors would be expected to occur in higher order oligomers.

*Trans*-phosphorylation initiates signaling by the PDGF  $\beta$  receptor and other RTKs by two mechanisms: (1) by augmenting the tyrosine kinase activity by removing inhibitory constraints, and (2) by generating binding sites for various SH2 domain-containing cellular signaling proteins [41]. Both events also appeared to occur upon E5-mediated receptor phosphorylation. As noted above, PDGF  $\beta$  receptor isolated from E5-transformed cells displayed increased *in vitro* tyrosine kinase activity, and various signal transduction proteins were constitutively associated with the receptor in these cells. Furthermore, velocity sedimentation has been used to isolate and study multiprotein signal transduction complexes containing activated PDGF  $\beta$  receptor, the E5 protein, and associated signal transduction proteins including PI3'kinase, PLC $\gamma$ , and ras-GAP [27].

### 4. Mutational analysis of the E5 protein/PDGF $\beta$ receptor interaction

Petti and Schaefer (Albany Medical College) have shown that the E5 protein can bind to a short fragment of the PDGF  $\beta$  receptor that contained only the transmembrane domain flanked by a few juxtamembrane amino acids, indicating that all the sequence information required for stable complex formation was localized to this fragment. Mutations in the E5 protein at the cysteines at positions 37 and 39, glutamine 17, or aspartic acid 33 prevented complex formation with the PDGF  $\beta$  receptor, receptor activation, and cell transformation, whereas receptor binding was not impaired by mutations at many other positions [12,15,28]. Similarly, mutations in the PDGF  $\beta$  receptor at transmembrane threonine 513 and juxtamembrane lysine 499 prevented complex formation and

transformation [40]. Mutations at the two E5 cysteines also prevented E5 dimer formation, suggesting that these mutants were transformation defective because they were unable to form dimers [12,15]. Some substitutions at glutamine 17 also affected the efficiency of E5 dimer formation, indicating that the residue at this position played a role in stabilizing the E5 dimer [14,30]. Schlegel et al. constructed and analyzed a series of alanine scanning mutations along the central hydrophobic domain of the E5 protein, and reported that substitutions at glutamine 17, leucine 21, and leucine 24 prevented complex formation with the PDGF  $\beta$  receptor [36].

When the juxtamembrane/transmembrane sequences of the E5 protein and the PDGF  $\beta$  receptor were aligned in an anti-parallel fashion (reflecting the opposite transmembrane orientation of these two proteins), we noted that the aspartic acid/lysine pair were juxtaposed, as were the glutamine/threonine pair (Fig. 4). This suggested that the E5/PDGF  $\beta$  receptor complex was stabilized largely by an electrostatic bond between the oppositely charged juxtamembrane lysine and aspartic acid 33 and by a hydrogen-bond or a packing interaction between the transmembrane threonine and glutamine 17 [15,28,30,32,40]. To test these hypotheses, we determined the phenotypes of E5 mutants containing each of the twenty amino acids at position 33 and at position 17 [30,32]. The results were striking: first, there was a near perfect correlation between the ability of various E5 mutants to bind to and activate the PDGF  $\beta$  receptor and to transform cells; second, all position 17 mutants unable to participate in hydrogen bonding were defective for complex formation, PDGF  $\beta$  receptor activation, and cell transformation; and third, a juxtamembrane negative charge on the E5 protein was required for all the three activities. These results provided strong support for the specific interactions proposed above and, by inference, for the existence of direct interactions between the E5 protein and the PDGF  $\beta$  receptor. Analysis of a more limited set of mutations at the two required positions in the

PDGF  $\beta$  receptor were consistent with these interpretations (unpublished results).

The E5 protein can bind to and activate the PDGF  $\beta$  receptor but not the PDGF  $\alpha$  receptor [20,22]. This specificity is conferred by the transmembrane/juxtamembrane region of the receptors [39]. Notably, the PDGF  $\alpha$  receptor lacks the essential transmembrane threonine and the essential juxtamembrane lysine. By replacing the wild-type amino acids in the PDGF  $\alpha$  receptor with lysine and threonine, it should be possible to test the model that the glutamine/threonine and aspartic acid/lysine pairs are crucial amino acids responsible for the E5/PDGF  $\beta$  receptor interaction. Of course, it is likely that some of the hydrophobic amino acids in the transmembrane domain of the E5 protein and the PDGF  $\beta$  receptor also participate in important packing interactions, but the available genetic results indicated that a specific amino acid sequence in this portion of the E5 protein was not required.

## 5. Models of the E5 protein/PDGF $\beta$ receptor interaction

We have developed models for the molecular interactions between monomers of the E5 protein in the E5 dimer and between dimeric E5 protein and the PDGF  $\beta$  receptor. Infrared spectroscopy provided evidence that chemically synthesized E5 dimers existed in lipid bilayers as pairs of transmembrane  $\alpha$ -helices organized as symmetric, parallel, left-handed coiled-coils [5]. Molecular dynamic simulations and energy minimizations identified two potential low energy structures of the E5 dimer [5]. In both structures, the E5 dimer is stabilized by hydrophobic interactions similar to a leucine zipper, and the aspartic acids point away from the dimer interface so that they are in position to form salt bridges with the essential lysines on the PDGF  $\beta$  receptor subunits. In one structure, the glutamines at position 17 lined the interface of the E5 dimer and formed hydrogen bonds across the interface, whereas

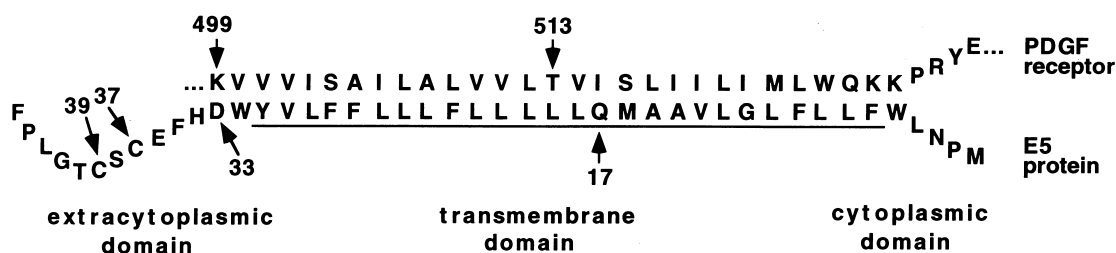


Fig. 4. Alignment of the sequences of the E5 protein and the transmembrane domain of the PDGF  $\beta$  receptor. The E5 protein and transmembrane region of the PDGF  $\beta$  receptor are shown in their anti-parallel orientation, and the putative transmembrane domain of the E5 protein is underlined. Note that the E5 sequence reads from right to left, and PDGF  $\beta$  receptor sequence from left to right. Residues known to be critical for complex formation between the two proteins are indicated by arrowheads. K represents lysine, T represents threonine, Q represents glutamine, D represents aspartic acid, and C represents cysteine. Modified from Ref. [32].

they faced out in the other structure. The genetic results demonstrating that the amino acid at position 17 played a role in dimerization of the E5 protein favors the structure with the glutamines projecting into the interface. In addition, the results of solid-state NMR experiments indicate that the glutamine does, in fact, form hydrogen bonds across the E5 dimer interface (Steven Smith, State University of New York, Stony Brook).

By considering possible arrangements of the E5 dimer and two molecules of the PDGF  $\beta$  receptor, we proposed that each face of the E5 dimer contains a binding site (i.e. one aspartic acid 33, one glutamine 17, and probably some intervening amino acids) that interacts with a molecule of the PDGF  $\beta$  receptor [5] (Fig. 5). Therefore, the E5 dimer contains two binding sites, one on each face, thus explaining how the E5

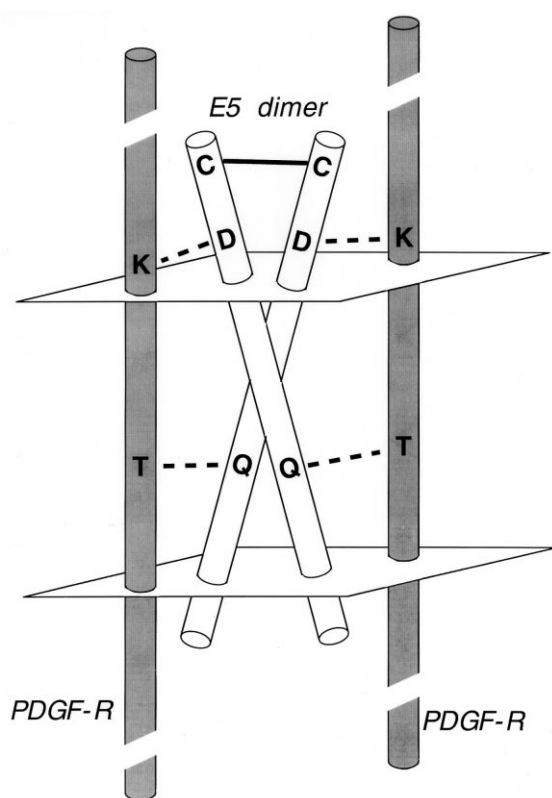


Fig. 5. Model for the interaction between the E5 protein and the PDGF  $\beta$  receptor. A model of the complex between a dimer of the E5 protein (open rods) and the transmembrane and juxtamembrane domains of two PDGF  $\beta$  receptor molecules (grey rods) is shown. The space bounded by the planes represents the cell membrane, with the cytoplasm at the bottom. The E5 protein and the PDGF  $\beta$  receptor are oriented in an anti-parallel fashion relative to one another. The solid line represents a disulfide bond between the cysteines in the E5 protein, and the dashed lines represent putative non-covalent bonds between residues important for complex formation. One feature of this model is that each PDGF  $\beta$  receptor molecule contacts both E5 monomers in the E5 dimer. Modified from Ref. [2].

dimer induces dimerization of the PDGF  $\beta$  receptor. We further proposed that on each face of the dimer the aspartic acid is contributed by one E5 monomer and the glutamine by the other. This arrangement accounts for the finding that the dimerization-defective E5 mutants were also defective for inducing receptor activation, because E5 dimerization was required to generate intact binding sites for the receptor. This model is supported by the molecular dynamic simulations of the E5 dimer, which showed that the aspartic acid of one monomer and the glutamine of the other were on the same face of the helix in the low energy structures [5]. Even in the preferred structure with the glutamine in the interface, it was possible to model the interaction so that side-chain functional groups of each glutamine can make hydrogen bonds to the PDGF  $\beta$  receptor threonine as well as to the other E5 monomer [5].

Taken together, our results suggest the following model of E5-induced PDGF  $\beta$  receptor activation: homodimerization of the E5 protein generates two binding sites for the PDGF  $\beta$  receptor, one on each face of the dimer. Two molecules of the PDGF  $\beta$  receptor simultaneously bind to each E5 dimer via interactions between specific transmembrane and juxtamembrane amino acids. This results in dimerization of the receptor and in the bringing of the two kinase domains into proximity so that they can carry out *trans*-phosphorylation. Phosphorylation of the receptor on a tyrosine in the activation loop further stimulates kinase activity, while phosphorylation of other tyrosines recruits and activates various SH2 domain-containing cellular signaling proteins.

The analysis of alanine scanning mutations indicated that only one face of the E5 helix was involved in activation of the PDGF  $\beta$  receptor and identified glutamine 17, leucine 21, and leucine 24 as forming the binding site for the receptor [36]. In the model based on studies from our laboratory, these amino acids were predicted to line the interface of the E5 dimer [5]. On the basis of their studies, Schlegel et al. proposed that each E5 monomer had an independent binding site for the PDGF  $\beta$  receptor, but the binding site was accessible only following E5 dimerization [36]. Thus, only dimeric E5 protein can bind to and activate the PDGF  $\beta$  receptor, leading to transformation. The model further proposed that some E5 mutants formed tetramers that could not bind to or activate the PDGF  $\beta$  receptor but were still able to transform cells by a different but unknown mechanism [36]. It is clear that additional studies are required to define the interactions between the E5 protein and the PDGF  $\beta$  receptor and to test these models. However, the fundamental feature of the proposed models is the same: dimerization of the E5 protein generates two binding sites for the PDGF  $\beta$  receptor, and receptor

dimerization and activation occurs when these two sites are occupied simultaneously.

## 6. Alternative targets of the E5 protein

The E5 protein also binds a number of additional cellular proteins, including the 16 kDa transmembrane subunit of the vacuolar H<sup>+</sup>-ATPase (V-ATPase), an enzyme which is responsible for controlling the pH of the Golgi apparatus and other intracellular organelles [38]. Association between the ATPase subunit and the E5 protein appears to be mediated largely by transmembrane interactions, with glutamine 17 of the E5 protein and a glutamic acid in the fourth transmembrane domain of the ATPase subunit playing critical roles in complex formation [42,43]. A ternary complex containing the E5 protein, the PDGF  $\beta$  receptor, and the V-ATPase subunit has been detected in COS cells overexpressing these proteins [38]. It has recently been demonstrated that Golgi acidification is impaired in cells transformed by the E5 protein, a response that appears to be due to inhibition of V-ATPase activity [44]. Because many important growth regulatory proteins, including the PDGF  $\beta$  receptor, transit through the Golgi apparatus en route to their final destination in the cell, it is possible that the ability of the E5 protein to perturb the pH of intracellular organelles may influence the activity of these proteins and contribute to transformation. It has also been reported that the E5 protein can associate with an  $\alpha$ -adaptin-like protein [45]. Since adaptins are involved in the metabolism of growth factor receptors, this interaction may also influence cell signaling.

It has been difficult to assess the role of the E5 protein/ATPase subunit interaction in cell transformation for several reasons. First, because it is difficult to detect the association of the E5 protein with the endogenous V-ATPase subunit, the great majority of studies examining the formation of a complex between these two proteins has been carried out in overexpressing cell systems. Second, because V-ATPase activity is essential for cell viability, V-ATPase null cells do not exist. Therefore, it has not been possible to carry out definitive gene transfer studies with wild type and mutant V-ATPase genes to determine their importance for E5 transformation. It was shown that a co-transfected V-ATPase subunit gene inhibited focus formation by the E5 gene, but it is not known if this reflected the restoration of normal V-ATPase activity in the cells or the sequestration of the E5 protein from other targets [42]. Third, there is no simple signature of V-ATPase activity in intact cells, comparable to tyrosine phosphorylation of the PDGF  $\beta$  receptor. Therefore, indirect measures of V-ATPase activity, such as measurement of Golgi pH, must be used.

Finally, it has not been possible to pharmacologically modulate V-ATPase activity and assess the effects of these treatments on E5 transformation. Despite these problems, studies in this area may reveal new aspects of E5-mediated transformation. In addition, because the human papillomavirus E5 proteins also interact with the V-ATPase subunit [46], study of this interaction may provide new insight into the pathogenesis of HPV-associated diseases, including cervical carcinoma.

In bovine warts, the E5 protein is expressed in dermal fibroblasts. In addition, the E5 protein is also expressed in basal epidermal keratinocytes and in some differentiated keratinocytes in these lesions, where it presumably plays a role in the productive virus life cycle [11]. Moreover, the E5 protein caused tumorigenic transformation of established murine keratinocytes [47]. Since keratinocytes do not normally express PDGF  $\beta$  receptor, these results suggested that in these cells the E5 protein interacted with other targets, such as the V-ATPase. Alternatively, it is possible that low levels of PDGF  $\beta$  receptor expression occurred in these cells and mediated the cellular response to the viral protein. Biochemical and genetic analysis is required to determine the mechanism of action of the E5 protein in these non-mesenchymal cell types.

## 7. What does the E5 protein tell us about growth factor receptor function?

The analysis of the E5 protein/PDGF  $\beta$  receptor interaction has defined a novel mechanism of DNA virus transformation. This analysis has also revealed new features of growth factor receptor signaling. Most importantly, these studies have established that receptor tyrosine kinases can be activated in a ligand-independent fashion by interactions with proteins that do not resemble their normal ligands. In the case of the PDGF  $\beta$  receptor, transmembrane and juxtamembrane amino acids participate in interactions with a “bridging” protein, resulting in receptor dimerization and activation. These results raise the possibility that short transmembrane proteins can be designed or selected that bind to the transmembrane domains of various growth factor receptors and other proteins. Some proteins identified in this fashion may cause dimerization and activation of their binding partners, whereas others may result in inhibition rather than activation. Such studies may not only lead to the generation of proteins with novel or useful properties, they may also help to define the molecular code that governs the association of protein transmembrane domains.

The nature of some of the biochemical events occurring during receptor activation has been elucidated through studies of the E5 protein/PDGF  $\beta$  receptor

system. Analysis of cells co-expressing the E5 protein and various receptor mutants has demonstrated that phosphorylation of the PDGF  $\beta$  receptor in the activated dimer occurs obligatorily in *trans* and that cellular signaling substrates are able to bind to hemi-phosphorylated receptor heterodimers containing kinase-active and -inactive receptor subunits [26,27]. The simultaneous presence of more than one signaling substrate in these complexes has also been established [27]. Finally, the use of velocity sedimentation in sucrose gradients has allowed the clear visualization of signal transduction complexes containing activated PDGF  $\beta$  receptor and associated signaling proteins [27]. These entities were first postulated over 10 years ago [48], but their detection, separation from inactive receptors, and characterization has proven surprisingly difficult.

An interesting activity of the E5 protein is its ability to activate intracellular forms of the PDGF  $\beta$  receptor. This activity is made evident by the constitutive tyrosine phosphorylation of intracellular PDGF  $\beta$  receptor in E5-transformed cells and the *trans*-phosphorylation of kinase-negative intracellular receptor in response to the E5 protein [18,26]. In addition, the precursor forms of the PDGF  $\beta$  receptor associated stably with various cellular signaling proteins and with the E5 protein itself, and these receptor forms were present in activated signal transduction complexes [23,27]. These results provided further support for the contention, first made with the *v-sis* oncogene product [49], that RTKs activated in intracellular membranes can generate a mitogenic signal. Indeed, analysis of an E5 mutant targeted to the endoplasmic reticulum suggested that Golgi location of E5 protein/PDGF  $\beta$  receptor complex was critical for transformation [50]. Further analysis of E5 transformation may provide new insights into the role of subcellular localization on signal transduction. Similarly, further analysis of the role of V-ATPase in E5 transformation may yield novel insights into regulation of growth factor receptor function.

The PDGF  $\beta$  receptor contains at least nine sites of tyrosine phosphorylation that mediate diverse effects, e.g., some sites stimulate kinase activity, others form docking sites for SH-2 or PTB domains, still others may be silent [39]. It is not known if all sites are phosphorylated on each receptor molecule, or if different molecules are phosphorylated at different sets of sites and the mapped sites represent the aggregate response. The stoichiometry of phosphorylation at individual tyrosines on the PDGF  $\beta$  receptor varies widely as a function of the length of ligand treatment [51]. Therefore, measuring global PDGF  $\beta$  receptor tyrosine phosphorylation by Western blotting with an antibody that recognizes phosphotyrosine may give an imperfect measure of receptor activation. In fact, although

ligand-induced RTK activation requires receptor dimerization and *trans*-phosphorylation, dimerization is not sufficient to induce mitogenic signaling. Rather, the correct rotational orientation of the receptor monomers within the dimer appear important for activity, perhaps because the orientation of subunits may determine the sites of phosphorylation [52–54]. Even if the PDGF  $\beta$  receptors in two activated complexes are phosphorylated on the same tyrosines, there may be differences in the spectrum of substrates bound or phosphorylated, thereby leading to differences in signal transduction. This would be the case if a particular arrangement of receptor molecules in a complex imposes steric constraints upon which substrates have access to their binding sites or which bound substrates can be phosphorylated, or if the phosphorylated receptors are in cellular locations where they engage different constellations of substrates.

These considerations may bear on some unresolved issues regarding the E5 protein/PDGF  $\beta$  receptor interaction. For example, there are E5 point mutants that underwent complex formation with the PDGF  $\beta$  receptor and caused receptor tyrosine phosphorylation, but did not transform C127 cells [28]. These mutants were also defective for inducing PDGF  $\beta$  receptor down-regulation, indicating that the interaction between the mutant E5 proteins and the PDGF  $\beta$  receptor was not normal. It is possible that these E5 mutants caused phosphorylation at only a subset of tyrosines and therefore induced qualitatively different receptor-initiated signals than did the wild-type E5 protein. Similarly, the relatively low levels of PDGF  $\beta$  receptor tyrosine phosphorylation induced by some E5 mutants that transform cells efficiently may reflect a low stoichiometry of phosphorylation at non-critical tyrosines [35,36].

Genetic analysis in Ba/F3 cells suggested that the E5 protein and *v-sis* utilized similar pathways to induce cell proliferation [23], and levels of PI3'kinase activity were elevated in E5-transformed fibroblasts [55]. However, the signal transduction pathways activated by the E5 protein are largely unexplored and not necessarily identical to those activated by treatment with PDGF. Indeed, the PDGF  $\beta$  receptor can be activated by diverse means — stimulation by PDGF (or expression of *v-sis*), expression of the E5 protein, substitution of the transmembrane domain from the activated allele of p185<sup>neu</sup>, mutation in the receptor cytoplasmic juxta-membrane domain, and fusion to dimerization domains of various cellular proteins [56–59]. This provides the opportunity to determine whether all activated PDGF  $\beta$  receptors generate the same signal in a given cellular context or whether the mechanism of activation dictates the signal output. If PDGF  $\beta$  receptor activated by these various means undergoes tyrosine phosphorylation at different constellations of sites or

permits different substrates to bind to these sites, then different outputs may result. The ability of a growth factor receptor to modulate its signal transduction cascade as a function of the means of activation would provide cells with a mechanism for fine-tuning the signaling pathways that are activated by related but non-identical stimuli.

## 8. Conclusion

Despite the detailed analysis of the E5 protein that has been carried out over many years, there are still a number of unanswered questions. Is PDGF  $\beta$  receptor activation the sole mechanism of E5-induced cell transformation? What does the E5 protein/PDGF  $\beta$  receptor interaction tell us about growth factor signaling in general? What are the precise molecular interactions that lead to complex formation between the E5 protein and its cellular targets? What is the role of V-ATPase in the biological activities of the E5 protein? What is the role of the E5 protein in the papillomavirus life-cycle and the pathogenesis of papillomavirus-induced diseases? Further study of these and related questions will continue to shed light on viral transformation and replication, cellular signal transduction, and the assembly of transmembrane protein complexes.

## Acknowledgements

We thank Lisa Petti and Steven Smith and their colleagues for permission to cite unpublished work, and we thank Jan Zulkeski for preparing this manuscript. The work in the authors' laboratory was supported by a grant from the National Institutes of Health (CA37157). DM is supported by a Bayer Predoctoral Fellowship.

## References

- [1] zur Hausen H. Viruses in human cancers. *Science* 1991;254:1167–73.
- [2] Drummond-Barbosa D, DiMaio D. Virocrine transformation. *Biochem Biophys Acta* 1997;1332:M1–M17.
- [3] DiMaio D, Lai CC, Klein O. Virocrine transformation: the intersection between viral transforming proteins and cellular signal transduction pathways. *Annu Rev Microbiol* 1998;52:397–421.
- [4] Schlegel R, Wade-Glass M, Rabson MS, Yang Y-C. The E5 transforming gene of bovine papillomavirus encodes a small hydrophobic protein. *Science* 1986;233:464–7.
- [5] Surti T, Klein O, Ascheim K, DiMaio D, Smith SO. Structural models of the bovine papillomavirus E5 protein. *Proteins: structure, function, and genetics* 1998;33:601–12.
- [6] DiMaio D, Guralski D, Schiller JT. Translation of open reading frame E5 of bovine papillomavirus is required for its transforming activity. *Proc Natl Acad Sci USA* 1986;83:1797–801.
- [7] Schiller JT, Vass WC, Vousden KH, Lowy DR. E5 open reading frame of bovine papillomavirus type 1 encodes a transforming gene. *J Virol* 1986;57:1–6.
- [8] Burkhardt A, DiMaio D, Schlegel R. Genetic and biochemical definition of the bovine papillomavirus E5 transforming protein. *EMBO J* 1987;6:2381–5.
- [9] Bergman P, Ustav M, Sedman J, Moreno-López J, Vennström B, Pettersson U. The E5 gene of bovine papillomavirus type 1 is sufficient for complete oncogenic transformation of mouse fibroblasts. *Oncogene* 1988;2:453–9.
- [10] Burkhardt A, Willingham M, Gay C, Jeang K-T, Schlegel R. The E5 oncoprotein of bovine papillomavirus is oriented asymmetrically in Golgi and plasma membranes. *Virology* 1989;170:334–9.
- [11] Burnett S, Jareborg N, DiMaio D. Localization of bovine papillomavirus type 1 E5 protein to transformed basal keratinocytes and permissive differentiated cells in fibropapilloma tissue. *Proc Natl Acad Sci USA* 1992;89:5665–9.
- [12] Horwitz BH, Burkhardt AL, Schlegel R, DiMaio D. 44-amino-acid E5 transforming protein of bovine papillomavirus requires a hydrophobic core and specific carboxyl-terminal amino acids. *Mol Cell Biol* 1988;8:4071–8.
- [13] Horwitz BH, Weinstat D, DiMaio D. Transforming activity of a sixteen-amino-acid segment of the bovine papillomavirus E5 protein linked to random sequences of hydrophobic amino acids. *J Virol* 1989;63:4515–9.
- [14] Kulke R, Horwitz BH, Zibello T, DiMaio D. The central hydrophobic domain of the bovine papillomavirus E5 transforming protein can be functionally replaced by many random hydrophobic sequences containing a glutamine. *J Virol* 1992;66:505–11.
- [15] Meyer AN, Xu Y-F, Webster MK, Smith AS, Donoghue DJ. Cellular transformation by a transmembrane peptide: structural requirements for the bovine papillomavirus E5 oncoprotein. *Proc Natl Acad Sci USA* 1994;91:4634–8.
- [16] Martin P, Vass W, Schiller JT, Lowy D, Velu TJ. The bovine papillomavirus E5 transforming protein can stimulate the transforming activity of EGF and CSF-1 receptors. *Cell* 1989;59:21–32.
- [17] Settleman J, Fazeli A, Malicki J, Horwitz BH, DiMaio D. Genetic evidence that acute morphologic transformation, stimulation of cellular DNA synthesis, and focus formation are mediated by a single activity of the bovine papillomavirus E5 protein. *Mol Cell Biol* 1989;9:5563–72.
- [18] Petti L, Nilson L, DiMaio D. Activation of the platelet-derived growth factor receptor by the bovine papillomavirus E5 protein. *EMBO J* 1991;10:845–55.
- [19] Nilson LA, DiMaio D. Platelet-derived growth factor receptor can mediate tumorigenic transformation by the bovine papillomavirus E5 protein. *Mol Cell Biol* 1993;13:4137–45.
- [20] Petti L, DiMaio D. Specific interaction between the bovine papillomavirus E5 protein and the platelet-derived growth factor receptor in stably transformed and acutely transfected cells. *J Virol* 1994;68:3582–92.
- [21] Petti L, DiMaio D. Stable association between the bovine papillomavirus E5 transforming protein and activated platelet-derived growth factor receptor in transformed mouse cells. *Proc Natl Acad Sci USA* 1992;89:6736–40.
- [22] Goldstein DJ, Li W, Wang L-M, Heidaran MA, Aaronson SA, Shinn R, Schlegel R, Pierce JH. The bovine papillomavirus type 1 E5 transforming protein specifically binds and activates the  $\beta$ -type receptor for platelet-derived growth factor but not other

- tyrosine kinase-containing receptors to induce cellular transformation. *J Virol* 1994;68:4432–41.
- [23] Drummond-Barbosa D, Vaillancourt RR, Kazlauskas A, DiMaio D. Ligand-independent activation of the platelet-derived growth factor  $\alpha$  receptor: requirements for bovine papillomavirus E5-induced mitogenic signaling. *Mol Cell Biol* 1995;15:2570–81.
- [24] Cohen BD, Goldstein DJ, Rutledge L, Vass WC, Lowy DR, Schlegel R, Schiller J. 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* 1993;67:5303–11.
- [25] Kulke R, DiMaio D. Biological activities of the E5 protein of the deer papillomavirus in mouse C127 cells: morphologic transformation, induction of cellular DNA synthesis and activation of the PDGF receptor. *J Virol* 1991;65:4943–9.
- [26] Lai CC, Henningson C, DiMaio D. Bovine papillomavirus E5 protein induces oligomerization and *trans*-phosphorylation of the platelet-derived growth factor  $\beta$  receptor. *Proc Natl Acad Sci USA* 1998;95:15241–6.
- [27] Lai CC, Henningson C, DiMaio D. Bovine papillomavirus E5 protein induces the formation of signal transduction complexes containing dimeric activated PDGF  $\beta$  receptor and associated signaling proteins. *J Biol Chem* 2000;275:9832–40.
- [28] Nilson LA, Gottlieb R, Polack GW, DiMaio D. 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* 1995;69:5869–74.
- [29] Kovalenko M, Gazit A, Böhmer A, Rorsman C, Rönstrand L, Heldin C-H, Waltenberger J, Böhmer F-D, Levitzki A. Selective platelet-derived growth factor receptor kinase blockers reverse *sis*-transformation. *Cancer Res* 1994;54:6106–14.
- [30] Klein O, Polack GW, Surti T, Kegler-Ebo D, Smith SO, DiMaio D. Role of glutamine 17 of the bovine papillomavirus E5 protein in platelet-derived growth factor  $\beta$  receptor activation and cell transformation. *J Virol* 1998;72:8921–32.
- [31] Riese DJ II, DiMaio D. An intact PDGF signalling pathway is required for efficient growth transformation of mouse C127 cells by the bovine papillomavirus E5 protein. *Oncogene* 1995;10:1431–40.
- [32] Klein O, Kegler-Ebo D, Su J, Smith S, DiMaio D. The bovine papillomavirus E5 protein requires a juxtamembrane negative charge for activation of the platelet-derived growth factor  $\beta$  receptor and transformation of C127 cells. *J Virol* 1999;73:3264–72.
- [33] Doolittle RF, Hunkapiller MW, Hood LE, Devare SG, Robbins KC, Aarason SA, Antoniades HN. Simian sarcoma virus *onc* gene *v-sis* is derived from the gene (or genes) encoding a platelet-derived growth factor. *Science* 1983;221:275–6.
- [34] Waterfield MD, Scrace GT, Whittle N, Stroobant P, Johnsson A, Wasteson A, Westermark B, Heldin C-H, Huang JS, Deuel TF. Platelet-derived growth factor is structurally related to the putative transforming protein p28<sup>sis</sup> of simian sarcoma virus. *Nature* 1983;304:35–9.
- [35] Sparkowski J, Mense M, Anders J, Schlegel R. E5 oncoprotein transmembrane mutants dissociate fibroblast transforming activity from 16-kilodalton protein binding and platelet-derived growth factor receptor binding and phosphorylation. *J Virol* 1996;70:2420–30.
- [36] Adduci AJ, Schlegel R. The transmembrane domain of the E5 oncoprotein contains functionally discrete helical faces. *J Biol Chem* 1999;274:10249–58.
- [37] Supryniewicz FA, Sparkowski J, Baega A, Schlegel R. E5 oncoprotein mutants activate phosphoinositide 3-kinase independently of platelet-derived growth factor receptor activation. *J Biol Chem* 2000;275:5111–9.
- [38] Goldstein DJ, Andresson T, Sparkowski JJ, Schlegel R. 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* 1992;11:4851–9.
- [39] Staebler A, Pierce JH, Brazinski S, Heidaran MA, Li W, Schlegel R, Goldstein DJ. 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* 1995;69:6507–17.
- [40] Petti LM, Reddy V, Smith SO, DiMaio D. Identification of amino acids in the transmembrane and juxtamembrane domains of the platelet-derived growth factor receptor required for productive interaction with the bovine papillomavirus E5 protein. *J Virol* 1997;71:7318–27.
- [41] Heldin C-H, Ostman A, Rönstrand L. Signal transduction via platelet-derived growth factor receptors. *Biochim Biophys Acta* 1998;1378:F79–F113.
- [42] Andresson T, Sparkowski J, Goldstein DJ, Schlegel R. Vacuolar H<sup>+</sup>-ATPase mutants transform cells and define a binding site for the papillomavirus E5 oncoprotein. *J Biol Chem* 1995;270:6830–7.
- [43] Goldstein D, Kulke R, DiMaio D, Schlegel R. A glutamine residue in the membrane-associating domain of the BPV-1 E5 oncoprotein mediates its binding to a transmembrane component of the vacuolar H<sup>+</sup>-ATPase. *J Virol* 1992;66:405–13.
- [44] Schapiro F, Sparkowski J, Adduci A, Supryniewicz F, Schlegel R, Grinstein S. Golgi alkalization by the papillomavirus E5 oncoprotein. *J Cell Biol* 2000;148:305–16.
- [45] Cohen BD, Lowy DR, Schiller J. 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* 1993;13:6462–8.
- [46] Conrad M, Bubb VJ, Schlegel R. The human papillomavirus type 6 and 16 E5 proteins are membrane-associated proteins which associate with the 16-kilodalton pore-forming protein. *J Virol* 1993;67:6170–8.
- [47] Leptak C, Ramon y Cajal S, Kulke R, Riese DR, Horwitz BH, Dotto GP, DiMaio D. Tumorigenic transformation of mouse keratinocytes by the E5 genes of human papillomavirus type 16 and bovine papillomavirus type 1. *J. Virol.* 65 1991 7078 7083. Correction: *J. Virol.* 66: 1833.
- [48] Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. *Cell* 1990;61:203–12.
- [49] Keating MT, Williams LT. Autocrine stimulation of intracellular PDGF receptors in *v-sis*-transformed cells. *Science* 1988;239:914–6.
- [50] Sparkowski J, Anders J, Schlegel R. E5 oncoprotein retained in the endoplasmic reticulum/cis Golgi still induces PDGF receptor autophosphorylation but does not transform cells. *EMBO J* 1995;14:3055–63.
- [51] Bernard A, Kazlauskas A. Phosphospecific antibodies reveal temporal regulation of platelet-derived growth factor  $\beta$  receptor signaling. *Exp Cell Res* 1999;253:704–12.
- [52] Burke CL, Lemmon MA, Coren BA, Engelman DM, Stern DF. Dimerization of the p185neu transmembrane domain is necessary but not sufficient for transformation. *Oncogene* 1997;14:687–96.
- [53] Burke CL, Stern DF. Activation of neu (ErbB-2) mediated by disulfide bond-induced dimerization reveals a receptor tyrosine kinase dimer interface. *Mol Cell Biol* 1998;18:5371–9.
- [54] Remy I, Wilson IA, Michnick SW. Erythropoietin receptor activation by a ligand-induced conformation change. *Science* 1999;283:990–3.

- [55] Ghai J, Ostrow RS, Tolar J, McGlennen RC, Lemke TD, Tobolt D, Liu Z, Faras AJ. The E5 gene product of rhesus papillomavirus is an activator of endogenous Ras and phosphatidylinositol-3'-kinase in NIH 3T3 cells. *Proc Natl Acad Sci USA* 1996;93:12879–84.
- [56] Carroll M, Tomasson MH, Barker GF, Golub TR, Gilliland DG. The TEL/platelet-derived growth factor  $\beta$  receptor (PDGF  $\beta$  R) fusion in chronic myelomonocytic leukemia is a transforming protein that self-associates and activates PDGF  $\beta$  R kinase-dependent signaling pathways. *Proc Natl Acad Sci USA* 1996;93:14845–50.
- [57] Irusta PM, DiMaio D. A single amino acid substitution in a WW-like domain of diverse members of the PDGF receptor subfamily of tyrosine kinases causes constitutive receptor activation. *EMBO J* 1998;17:6912–23.
- [58] Petti LM, Irusta PM, DiMaio D. Oncogenic activation of the PDGF  $\beta$  receptor by the transmembrane domain of p185<sup>neu\*</sup>. *Oncogene* 1998;16:843–51.
- [59] Ross TS, Bernard OA, Berger R, Gilliland DG. Fusion of Huntington interacting protein 1 to platelet-derived growth factor  $\beta$  receptor (PDGF $\beta$ R) in chronic myelomonocytic leukemia with t(5;7) (q33; q11.2). *Blood* 1998;91:4419–26.