Specific Interaction between the Bovine Papillomavirus E5 Transforming Protein and the β Receptor for Platelet-Derived Growth Factor in Stably Transformed and Acutely Transfected Cells

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The E5 protein of bovine papillomavirus is a 44-amino-acid membrane protein which induces morphologic and tumorigenic transformation of fibroblasts. We previously showed that the E5 protein activates and forms a complex with the endogenous β receptor for platelet-derived growth factor (PDGF) in transformed rodent fibroblasts and that the PDGF β receptor can mediate tumorigenic transformation by the E5 protein in a heterologous cell system. Other workers have identified the receptor for epidermal growth factor (EGF) as a potential target of the E5 protein in NIH 3T3 cells. Here, we investigate the specificity of the interaction of the E5 protein with various growth factor receptors, with particular emphasis on the PDGF β receptor and the EGF receptor. Under conditions where both the PDGF β receptor and the EGF receptor are stably expressed in E5-transformed mouse and bovine fibroblasts and in E5-transformed epithelial cells, the E5 protein specifically forms a complex with and activates the PDGF receptor and not the EGF receptor. Under conditions of transient overexpression in COS cells, the E5 protein has the potential to associate with several growth factor receptors, including the EGF receptor. However, upon coexpression of PDGF β receptors and EGF receptors in COS cells, the E5 protein preferentially forms a complex with the PDGF receptor. Therefore, we conclude that the PDGF β receptor is the primary target for the E5 protein in a variety of cell types, including bovine fibroblasts.

Although most papillomaviruses induce the proliferation of epithelial cells, a subset, the fibropapillomaviruses, induce the proliferation of dermal fibroblasts as well. The prototype fibropapillomavirus, bovine papillomavirus type 1 (BPV), induces skin fibropapillomas and other mesenchymal tumors in infected animals. Like the other fibropapillomaviruses, BPV readily induces oncogenic transformation of cultured rodent fibroblasts, and most of this activity resides in the 44-amino-acid E5 protein (2, 3, 10, 43). Expression of the E5 protein alone in rodent fibroblasts is sufficient to induce morphologic and tumorigenic transformation (2, 28, 46), stimulate quiescent cells to undergo DNA synthesis (19, 23, 46), and augment the transforming activity of a variety of transfected genes, including some that encode cell surface growth factor receptors (13, 30, 51). This protein is also thought to be responsible for fibroblast proliferation in fibropapilloma formation. The E5 protein exists as a homodimer with a subunit size of 7 kDa and localizes primarily to intracytoplasmic membranes in BPV-transformed cells (4, 5, 44). The amino-terminal two-thirds of the E5 protein is extremely hydrophobic, while the carboxy-terminal third is hydrophilic. Mutational analysis in C127 cells has identified eight amino acids that are required for the transforming activity of the E5 protein, seven located in the carboxy-terminal hydrophilic domain, and one, a glutamine, located in the hydrophobic domain (21, 22, 25, 46). In addition, the overall hydrophobicity of the E5 protein is essential for transformation (22, 25).

Because only a few specific amino acids are required for the transforming activity of the E5 protein, it is unlikely that it

possesses an intrinsic enzymatic activity. Instead, we proposed that the E5 protein, like other DNA tumor virus transforming proteins, acts indirectly by modulating the activity of a cellular protein involved in the control of normal cell growth (21). In support of this model, we showed that the E5 protein induces constitutive activation of a transmembrane growth factor receptor tyrosine kinase, the endogenous β receptor for platelet-derived growth factor (PDGF), in transformed rodent fibroblasts (34). Furthermore, the E5 protein and activated forms of the PDGF receptor exist in a stable complex in transformed cell lines, implying that receptor activation is induced by a physical association between the E5 protein and the PDGF receptor (33). In several cell types, an immature membrane-associated form of the PDGF receptor is preferentially associated with and activated by the E5 protein (31, 33, 34). Moreover, using gene transfer experiments into cells lacking PDGF receptors, we have shown that the PDGF β receptor can deliver the transforming signal induced by the E5 protein (31). These results provide compelling evidence that the PDGF β receptor is an important cellular target for the E5 protein. It is not known whether the E5 protein and the PDGF receptor interact directly or whether they are components of a larger multiprotein complex. It has been suggested that another cellular protein, a 16-kDa subunit of the vacuolar H^+ -ATPase (16K), mediates the association of the E5 protein to the PDGF receptor since it interacts with both proteins under conditions of overexpression in COS cells (14). However, the evidence that the E5 protein, 16K, and the PDGF receptor exist in a single ternary complex is indirect, and the biochemical and physiological consequences of the E5-16K interaction are not known (14–16).

Others have proposed that the receptor for epidermal growth factor (EGF) is an important cellular target for the E5

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protein. Martin et al. reported that the E5 protein can stimulate the transforming activity of the human EGF receptor in NIH 3T3 cells and affect the metabolism of this receptor in these cells (30). Specifically, E5 expression was reported to result in increased half-life of the EGF receptor and in decreased receptor internalization in response to ligand (30). However, subsequent studies suggested that the major effect of E5 expression on the EGF receptor is to stabilize receptor-ligand interactions (50). Moreover, there appears to be an increase in tyrosine phosphorylation of transfected EGF receptors in E5-transformed NIH 3T3 cells, and complex formation between the E5 protein and the human EGF receptor has been detected when both proteins are overexpressed in COS cells (8, 30). Here we directly compared the ability of the E5 protein to form a complex with and activate the PDGF β receptor and the EGF receptor coexpressed in three independently derived, E5-transformed cell lines, including bovine fibroblasts, a natural host cell of BPV. We showed that the E5 protein specifically interacts with the PDGF receptor and not the EGF receptor in all three cell lines. In addition, we showed that although the E5 protein is capable of interacting with several growth factor receptors, including the EGF receptor, in COS cells, it preferentially forms a complex with PDGF receptors and not EGF receptors when both receptors are coexpressed in COS cells.

MATERIALS AND METHODS

Stable cell lines. A C127 cell line (CER-1) expressing the human EGF receptor gene was established by calcium phosphate-mediated DNA transfection as described previously (10), using 2 μ g of pCO11-EGFR-neo (gift from D. Lowy) (49) followed by isolation of individual G418-resistant colonies. A BPV-transformed C127 cell line expressing the human EGF receptor gene (CERE5-12) was established by cotransfection of 2 μ g of pCO11-EGFR-neo and 30 ng of *Bam*HI-digested pBPV-142.6 (wild-type BPV) (42) followed by isolation of individual morphologically transformed, G418-resistant colonies. CER-1 and CERE5-12 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics (DMEM-10). Normal bovine conjunctival fibroblasts (Con-12) and derivative cells (B2) transformed by infection with purified BPV-1 virions were the gift of S. Burnett (Uppsala, Sweden) (5) and maintained in DMEM-10. Normal NMuMG cells (3Y8) and derivative cells expressing the mouse PDGF β receptor (3B4) or the mouse PDGF β receptor and the BPV E5 protein (3A9) were described previously and were the gift of L. Nilson, Yale University (31).

Transient expression in COS cells. COS 7 cells were obtained from J. Settleman (Harvard University) and maintained in DMEM supplemented with 10% calf serum (DMEM-10C). Calcium phosphate-mediated DNA transfections were performed on confluent COS cell monolayers as described for C127 cells except that cells were subjected to a 1-min glycerol shock (using 15% glycerol in HEBS buffer) followed by 5 mM sodium butyrate treatment for 12 to 16 h (31). The medium was then replaced with fresh DMEM-10C, and 24 h later, the cells were either lysed directly or metabolically labeled and then lysed for immunoprecipitation as described below. The following DNAs were used for COS cell transfections. pPRCHH-8 (gift from C.-H. Heldin, Uppsala, Sweden) (7) and pSVR1 (gift from L. T. Williams, University of California, San Francisco) (12, 52) contain the human and mouse PDGF receptor genes, respectively, cloned into the pSV7d vector. pSR α -EGFR (subcloned by L. Du, L. Petti, and D. DiMaio

from pSKEGFR; a gift from D. Stern, Yale University) (49), pSR α -neuN (gift from D. Stern, Yale University) (1, 6), pSR α -CSF-1R (subcloned from pSMc-*fms*, a gift from C. Sherr, St. Jude's Children's Hospital) (11, 41), and pSR α -G (gift from J. Rose, Yale University) (40) contain the human EGF receptor gene, rat *c-neu*, human *c-fms*, and the vesicular stomatitis virus (VSV) G protein (VSV-G) genes, respectively, cloned into the SR α expression vector (48). pSV7d-CSFR contains the colony-stimulating factor 1 (CSF-1) receptor gene subcloned into the pSV7d vector (11). pMT2E5 contains the BPV E5 gene subcloned into the pMT2 expression vector (20).

Antibodies. Rabbit anti-PDGF receptor antiserum (α -PR4) was raised against a peptide containing the 13 C-terminal amino acids of the human β receptor (gift from S. Courtneidge, Heidelberg, Germany [26], and L. Nilson, Yale University). Rabbit antiserum 06-216, which specifically recognizes the C terminus of the human α PDGF receptor, was purchased from Upstate Biotechnology, Inc. (UBI). An anti-EGF receptor mouse monoclonal antibody (clone LA22) specific for amino acids 351 to 364 of the human EGF receptor was obtained from UBI and used only in Fig. 4. For all other experiments requiring an EGF receptor antibody, sheep polyclonal antiserum 06-129 (UBI) recognizing the cytoplasmic domain of the human and mouse EGF receptor was used. Control experiments demonstrated that this antiserum efficiently immunoprecipitates EGF receptor from bovine cells as well. Anti-p185^{neu} monoclonal antibodies Ab3 (clone 3B5) and Ab4 (clone 7.16.4) were obtained from Oncogene Science, Inc., and were used for immunoblotting and immunoprecipitation, respectively. A rabbit anti-CSF-1 receptor antiserum (4069) specific for the kinase insert domain of murine CSF-1 receptor was the gift of L. Rohrschneider (Fred Hutchinson Cancer Institute) (24, 38). A rabbit antiserum (6647), which was raised against VSV and recognizes VSV-G, was a gift of J. Rose (Yale University) (39). Antiphosphotyrosine monoclonal antibody 4G10 was purchased from UBI. An anti-E5 rabbit antiserum was raised to the 16 C-terminal amino acids of the BPV E5 protein (5).

Analysis of metabolically labeled cells. Confluent cells in 6-cm-diameter (COS cells) or 10-cm-diameter (bovine cells) dishes were washed twice with phosphate-buffered saline (PBS), incubated for 30 min in serum- and cysteine-free DMEM, and then incubated in fresh serum- and cysteine-free medium containing 0.2 mCi of [³⁵S]cysteine (48 TBq/mmol; Amersham) per ml for 5 h at 37°C. In Fig. 6C, cells were labeled for 5 h in serum-, cysteine-, and methionine-free DMEM containing 0.2 mCi of PROMIX, a mixture of 70% [³⁵S]methionine and 30% [³⁵S]cysteine (37 TBq/mmol; Amersham), per ml. Cells were washed twice with PBS containing phenylmethylsulfonyl fluoride (PMSF), scraped off the dish, pelleted, and frozen at -70°C overnight.

Cell pellets were thawed and then lysed in 200 μ l (Fig. 4) or 500 μ l (Fig. 1B) of cold radioimmunoprecipitation assay (RIPA) buffer (20 mM morpholinepropanesulfonic acid [MOPS], 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 1 mM PMSF) or 60 μ l of cold EBC buffer (50 mM Tris-Cl [pH 8], 120 mM NaCl, 0.5% Nonidet P-40, 1 mM PMSF) (Fig. 6C). After incubation on ice for 10 min, identical lysates were pooled and cleared of cell debris by centrifugation.

In the experiment shown in Fig. 1B, 150 μ l of labeled B2 cell extract was immunoprecipitated with 11 μ l of the α -PR4 antiserum or 5 μ l of the α -EGFR (06-129) antiserum, and 55 μ l of labeled extract was immunoprecipitated with 5 μ l of the α -E5 antiserum as previously described (34). Immune complexes were eluted from the beads by boiling in RIPA buffer

containing 1% SDS and as previously described (33) and subjected to a second round of immunoprecipitation with 5 μ l of the E5 antiserum. The washed immune complexes were resuspended in 60 μ l of 2 $\times$ Laemmli sample buffer and then electrophoresed on an SDS–8 to 20% polyacrylamide gel.

In the experiment shown in Fig. 4, extracts containing equal amounts of incorporated counts were immunoprecipitated with saturating amounts of receptor antisera, i.e., 6 μ l of α -p185^{neu} (Ab4), 2 μ l of α -CSF-1 receptor (4069), 2.5 μ l of α -PR4, 6 μ l of α -EGF receptor (LA22), or 3 μ l of normal rabbit serum. The E5 protein was also directly immunoprecipitated by adding 1.5 μ l of the α -E5 antiserum to extracts containing one-fifth the incorporated counts used for the receptor immunoprecipitations. Immune complexes were washed, resuspended in 50 to 65 μ l of 2 $\times$ Laemmli sample buffer, boiled for 5 min, and then electrophoresed on an SDS–10 to 20% polyacrylamide gel.

In the experiment shown in Fig. 6C, extracts were precleared with 5 μ l of normal rabbit serum, and equal amounts of incorporated counts were immunoprecipitated with 1 μ l of normal rabbit serum, 1 μ l of the α -PR4 antiserum, or 1 μ l of the α -EGFR (06-129) antiserum. Washed immune complexes were resuspended in 20 μ l of 2 $\times$ Laemmli sample buffer and electrophoresed on an SDS–7.5% polyacrylamide–0.17% bisacrylamide gel. Standard procedures were used to process the gels for fluorography, using Amplify (Amersham).

Immunoprecipitation from unlabeled cell extracts. In Fig. 2 and 3 and lane 7 of Fig. 1A, cell extracts were prepared as described above for metabolically labeled extracts. In Fig. 5, 6A, and 6B and the remaining lanes of Fig. 1A, cells were lysed directly in the dish by incubating in cold RIPA or cold EBC buffer for 20 min on ice as previously described (34).

Coimmunoprecipitation analysis was performed as described above, using approximately 3 to 5 μ l of the α -E5 antiserum per mg of extracted protein from bovine, C127, or NMuMG cells or 10 to 15 μ l of the α -E5 antiserum per mg of COS cell extract. In Fig. 1A and 5B, anti-E5 immune complexes were eluted from the protein A-Sepharose beads by boiling in RIPA buffer containing 1% SDS, diluted to 0.1% SDS in RIPA buffer, and incubated for 2 h with 100 μ l of a 1:1 suspension of wheat germ lectin-Sepharose in RIPA buffer (WGA) (Fig. 5B) or a mixture of WGA and lentil lectin-Sepharose (Fig. 1A) to precipitate glycoproteins associated with the E5 protein. The various receptors were immunoprecipitated directly or affinity purified by using 1.5 μ l of α -PR4, 0.5 μ l of α -EGF receptor (06-129), 2 μ l of α -p185^{neu}, 5 μ l of α -VSV-G, or 100 μ l of WGA (for CSF-1 receptor) per 100 μ g of extracted protein.

Immunoblotting. Washed immunoprecipitates were boiled in 2 $\times$ Laemmli sample buffer, electrophoresed on SDS–7.5% polyacrylamide–0.17% bisacrylamide or SDS–10% polyacrylamide–0.27% bisacrylamide gels, and transferred electrophoretically to nitrocellulose at 50 V overnight or 100 V for 1 to 2 h as previously described (34). Phosphotyrosine immunoblot analysis was performed with a 1:500 dilution of antiphosphotyrosine monoclonal antibody 4G10 as previously described (33) except that the immunoblot shown in Fig. 1A was subjected to enhanced chemiluminescence (ECL) detection as described by the manufacturer (Amersham), using a 1:5,000 dilution of protein A conjugated to horseradish peroxidase (HRP) (Amersham). The PDGF receptor immunoblot shown in Fig. 5A was processed as previously described (33), using a 1:250 dilution of the α -PR4 antiserum. All other PDGF receptor immunoblots were processed similarly except that the rinsing buffer was TNET (10 mM Tris, 50 mM NaCl, 2.5 mM EDTA, 0.1% Tween 20 [pH 7.4]), the blocking buffer was

MBB-1 (5% nonfat dry milk in TNET), and ECL detection was used after incubation with protein A-HRP in MBB-1 as described above. EGF receptor, CSF-1 receptor, and p185^{neu} immunoblots were all blocked in MBB-2 (5% nonfat dry milk in TBST [10 mM Tris-Cl {pH 7.4}, 150 mM NaCl, 0.05% Tween 20]) and washed in TBST throughout. EGF receptor immunoblots were incubated in a 1:1,000 dilution of anti-EGF receptor antiserum 06-129, washed, incubated in a 1:1,000 dilution of rabbit anti-sheep immunoglobulin G secondary antibody (Pierce), washed again, and detected with [¹²⁵I]protein A as previously described (34). The CSF-1 receptor immunoblot shown in Fig. 5B was incubated in a 1:1,000 dilution of anti-CSF-1 receptor antiserum 4069 and processed for ECL detection using protein A-HRP as described above. The p185^{neu} immunoblot shown in Fig. 5C was incubated with a 1:50 dilution of anti-p185^{neu} monoclonal antibody Ab3 and processed for ECL detection as described above except that a 1:10,000 dilution of a anti-mouse immunoglobulin G secondary antibody conjugated to HRP (Pierce) was used. The immunoblot shown in Fig. 5D was processed as previously described for antiphosphotyrosine blotting (34) except that a 1:100 dilution of the anti-VSV antiserum was used.

RESULTS

Target specificity of the E5 protein in stably transformed cells. To assess the specificity of the interaction between the E5 protein and growth factor receptors in stably transformed cells, we attempted to detect an interaction between the E5 protein and a tyrosine kinase growth factor receptor other than the PDGF β receptor. We chose to focus on the ability of the EGF receptor to interact with the E5 protein, since it has been reported that the E5 protein affects the activity and metabolism of transfected human EGF receptors in NIH 3T3 cells (8, 30) and that it can form a complex with the human EGF receptor in COS cells (8).

The target specificity of the E5 protein was first assessed in bovine fibroblasts, one of the natural host cells of BPV. These cells were transformed by infection with BPV virions to most closely approximate a natural infection. We determined the tyrosine phosphorylation status of endogenous growth factor receptors in normal or BPV-transformed cells by immunoprecipitating PDGF receptor and EGF receptor with specific antisera followed by antiphosphotyrosine immunoblot analysis (Fig. 1A). There is a low basal level of tyrosine phosphorylation of the PDGF β receptor in normal cells (Fig. 1A, lane 1) which is markedly increased in the BPV-transformed bovine fibroblasts (lane 2). The increase in tyrosine-phosphorylated PDGF receptor observed in the transformed cells is not due to an increase in total receptor amounts (data not shown). The faster-migrating form of tyrosine phosphorylated PDGF β receptor in transformed cells corresponds to an immature form of the PDGF receptor that is activated by the E5 protein in a variety of cell types (31, 34). In contrast, although the EGF receptor antibody readily precipitates the bovine EGF receptor from these cells (data not shown), no tyrosine-phosphorylated EGF receptor could be detected regardless of E5 expression (lane 6). Therefore, the endogenous PDGF β receptor and not the endogenous EGF receptor is activated by the E5 protein in BPV-transformed bovine fibroblasts. In addition, by using an antibody which specifically immunoprecipitates the α form of the bovine PDGF receptor (data not shown), a low level of tyrosine-phosphorylated PDGF α receptor can be detected in normal and BPV-transformed bovine fibroblasts, with little if any increase in PDGF α receptor tyrosine phosphorylation in response to E5 expression (lanes 3 and 4).

FIG. 1. Target specificity of the BPV E5 protein in bovine fibroblasts. (A) Extracts from normal (N) or BPV-transformed (B) bovine fibroblasts were immunoprecipitated (IP) with one of the following antisera as indicated: α -PR4 (PR), which recognizes both α and β forms of the PDGF receptor; 06-216 (PR α), which recognizes only the α form of the PDGF receptor; 06-129 (ER), which recognizes the EGF receptor; or α -E5 (E5), which is specific for the BPV E5 protein. In lane 7, glycoproteins were affinity purified from denatured E5 immune complexes by precipitation with lectin-Sepharose beads as described in Materials and Methods. Immunoprecipitates and the lectin precipitate were electrophoresed and then immunoblotted with an antiphosphotyrosine antibody. Each lane represents approximately 115 μ g of extracted protein, except for lane 7, which represents 1,500 μ g of protein. Arrowheads on the right indicate the positions of mature (more slowly migrating) and precursor forms of the PDGF β receptor. (B) Extracts from metabolically labeled BPV-transformed bovine fibroblasts were immunoprecipitated with either the E5, ER, or PR antiserum used in panel A. In lanes 1 and 4, antisera were preincubated with an excess of competing E5 and PDGF receptor peptides, respectively. Immune complexes were denatured and subjected to a second round of immunoprecipitation using the E5 antiserum and then electrophoresed as described in Materials and Methods. Lanes 3 to 5 each represent approximately 2.7-fold more incorporated counts than lane 1 or 2. The arrowhead on the right indicates the position of the E5 protein. Numbers on the left in panels A and B indicate positions of molecular weight size markers in kilodaltons.

Coimmunoprecipitation analysis was used to compare the ability of the E5 protein to form stable complexes with the PDGF β receptor and the EGF receptor in BPV-transformed bovine fibroblasts. First, antireceptor antisera were tested for the ability to coimmunoprecipitate the E5 protein (Fig. 1B). Anti-PDGF receptor and anti-EGF receptor immune complexes from extracts of metabolically labeled cells were denatured and subjected to a second round of immunoprecipitation using the anti-E5 antiserum. The E5 protein was detected by electrophoresis followed by autoradiography. It is evident that the E5 protein is present in the PDGF receptor immune complexes but not in the EGF receptor immune complexes (lane 5 versus lane 3). Coimmunoprecipitation of the E5 protein by the PDGF receptor antiserum was blocked with the immunizing PDGF receptor peptide (lane 4), ruling out the possibility that the E5 protein is nonspecifically precipitated by this antiserum. These results indicate that the E5 protein forms a stable complex with and activates the PDGF β receptor and not the EGF receptor in transformed bovine fibroblasts. This conclusion was confirmed in an experiment in which an immunoprecipitate generated with an anti-E5 antiserum was subjected to Western immunoblot analysis using a phosphotyrosine antibody. Two phosphotyrosine-containing proteins which comigrate with tyrosine-phosphorylated mature and precursor forms of the PDGF β receptor can be detected in E5 immunoprecipitates (Fig. 1A, lane 7), indicating that the activated forms of the PDGF β receptor form a stable complex with the E5 protein in bovine fibroblasts.

Approximately 10-fold more PDGF β receptor than EGF receptor is expressed in the bovine fibroblasts (data not shown). Therefore, it is possible that the apparent specific interaction of the E5 protein with the PDGF receptor in these cells reflects the relative amounts of these receptors rather than a true difference in the binding affinity of the E5 protein for these receptors. On the other hand, it is also possible that BPV and other fibropapillomaviruses have evolved to express a transforming protein which binds specifically to the PDGF β receptor, thereby inducing receptor activation. To distinguish between these two possibilities, the ability of the E5 protein to discriminate between the PDGF receptor and the EGF receptor was examined in murine cell lines expressing similar levels of both receptors.

First, the target specificity of the E5 protein was examined in mouse C127 fibroblasts, the cell line most frequently used to study BPV transformation. Although C127 cells express high levels of endogenous PDGF β receptors (Fig. 2A, lanes 1 to 3), these cells express low levels of endogenous EGF receptors (Fig. 2B, lane 3, and data not shown). Therefore, C127 cells were transfected with a plasmid expressing the human EGF receptor gene or cotransfected with separate plasmids expressing full-length BPV and the human EGF receptor, and stable cell lines were established. The cells expressing the E5 protein were morphologically transformed. Human EGF receptor expression in the resulting cell lines was confirmed by immunoblot analysis (Fig. 2B, lanes 1 and 2) and by immunoprecipitation of metabolically labeled cells (data not shown). In the BPV-transformed cells derived in this fashion, approximately 1.5-fold more β PDGF receptor than EGF receptor is expressed, as assessed by using saturating amounts of specific antibody to immunoprecipitate the receptors from metabolically labeled cells (data not shown). L1D-1, a previously established and characterized E5-transformed C127 cell line (34), was also analyzed as a control. To determine whether expression of the E5 protein results in the activation of both EGF and PDGF receptors in C127 cells, anti-PDGF receptor, anti-EGF receptor, and anti-E5 immunoprecipitates were sub-

FIG. 2. Target specificity of the BPV E5 protein in C127 mouse cells. Extracts of C127 cells expressing the human EGF receptor gene (ER) and/or the BPV E5 gene, as indicated, were prepared. Cell line CER-1 was analyzed in lanes 1, 4, and 7; cell line CERE5-12 was analyzed in lanes 2, 5, and 8; and cell line L1D-1 was analyzed in lanes 3, 6, and 9. These extracts were immunoprecipitated with either the anti-PDGF receptor antiserum PR4 (α -PR), the anti-EGF receptor antiserum (α -ER), or the anti-E5 antiserum (α -E5), as indicated. In panels A and B, lanes 7 to 9, immunoprecipitations were performed after the E5 antiserum was preincubated with an excess of immunizing E5 peptide (P). Immunoprecipitates were subjected to immunoblot analysis using α -PR (A), α -ER (B), or an antiphosphotyrosine antibody (C). In panels A and B, lanes 1 to 3 represent 100 (A) and 50 (B) μ g of extracted protein, while lanes 4 to 9 represent 975 μ g of extracted protein. In panel C, the amounts of extracted protein used per lane were 135 μ g (lanes 1 to 3 and 6), 170 μ g (lane 4), 107 μ g (lane 5), 2,460 μ g (lane 7), and 2,100 μ g (lanes 8 and 9). Arrowheads on the

jected to antiphosphotyrosine immunoblotting (Fig. 2C). Tyrosine phosphorylation of the precursor form of the endogenous PDGF receptor is induced by expression of the E5 protein in C127 cells even if the cells express human EGF receptor (Fig. 2C, lane 2 compared with lane 1). In contrast, no tyrosine-phosphorylated EGF receptor can be detected in cells expressing E5 and EGF receptor (Fig. 2C, lane 5), although abundant tyrosine-phosphorylated EGF receptor is detectable upon EGF treatment (data not shown). Furthermore, coimmunoprecipitation with the E5 antiserum followed by antiphosphotyrosine Western blotting indicates that the tyrosine-phosphorylated PDGF receptor is in a complex with the E5 protein (Fig. 2C, lane 8). Therefore, although abundant amounts of both PDGF receptors and EGF receptors are produced in these cells, the E5 protein specifically interacts with and activates the PDGF β receptor. Similar results were observed in additional C127 cell lines which express the BPV E5 protein and the human EGF receptor (31a).

Although the E5 protein does not appear to activate the EGF receptor in C127 cells, it is possible that the E5 protein and unphosphorylated EGF receptor form a complex in these cells. The ability of the E5 protein to bind to the EGF receptor and the PDGF β receptor was assessed by coimmunoprecipitation using the anti-E5 antiserum followed by anti-PDGF receptor (Fig. 2A) or anti-EGF receptor (Fig. 2B) immunoblotting. As expected, both mature and precursor PDGF β receptor forms can be coimmunoprecipitated from extracts of E5-transformed cells, whether or not they express exogenous EGF receptor (Fig. 2A, lanes 5 and 6). Little, if any, PDGF receptor is detected in E5 immunoprecipitates from extracts of untransformed cells (Fig. 2A, lane 4), and coimmunoprecipitation of the PDGF receptor from extracts of transformed cells is blocked by preadsorption of the E5 antiserum with the immunizing E5 peptide (Fig. 2A, lanes 8 and 9). Therefore, the presence of PDGF receptor in the E5 immunoprecipitates signifies complex formation between the E5 protein and the PDGF receptor rather than antiserum cross-reactivity. In contrast, EGF receptor immunoblotting failed to reveal EGF receptor in a complex with the E5 protein in extracts of C127 cells expressing the E5 protein and the human EGF receptor (Fig. 2B, lane 5), even though abundant amounts of EGF receptor are present in these extracts (Fig. 2B, lane 2). Therefore, although these cells express comparable levels of both PDGF receptors and EGF receptors (Fig. 2A and B, lanes 2, and data not shown), the E5 protein specifically interacts with and activates the PDGF receptor.

The binding specificity of the E5 protein was also examined in a mouse epithelial cell line, NMuMG, which normally express moderately high levels of endogenous EGF receptors but no endogenous PDGF β receptors (31). Expression of the E5 protein in NMuMG cells can render these cells tumorigenic only if they also express exogenous PDGF β receptors, providing strong genetic evidence that the PDGF β receptor can mediate transformation by the E5 protein (31). We previously reported that coexpression of the E5 protein and the PDGF β receptor in NMuMG cells resulted in the tyrosine phosphorylation of the PDGF receptor precursor and the association of this activated receptor form with the E5 protein (31). However, E5 expression did not result in tyrosine phosphorylation of the endogenous EGF receptor (31), suggesting that the PDGF

left indicate positions of the mature and precursor form of the PDGF receptor (A) and the EGF receptor (B). Sizes in kilodaltons are indicated on the right.

FIG. 3. Target specificity of the E5 protein in NMuMG murine epithelial cells. Extracts of normal NMuMG cells (3Y8), derivative cells expressing the PDGF receptor (3B4), or cells coexpressing the PDGF receptor and the E5 gene (3A9) were prepared. Cell line 3Y8 was analyzed in lanes 1 and 4; cell line 3B4 was analyzed in lanes 2 and 5; and cell line 3A9 was analyzed in lanes 3 and 6. These extracts were immunoprecipitated with either the anti-PDGF receptor (α -PR), the anti-EGF receptor (α -ER), or the anti-E5 (α -E5) antiserum as indicated. Immunoprecipitates were subjected to immunoblot analysis using the α -PR (A) or the α -ER (B) antiserum. Lanes 1 to 3 represent 200 (A) or 100 (B) μ g of extracted protein, while lanes 4 to 6 of both panel A and panel B represent 2,000 μ g of extracted protein. Arrowheads on the left indicate positions of mature and precursor forms of the PDGF receptor (A) and the position of the EGF receptor (B). Sizes in kilodaltons are indicated on the right.

receptor is the preferred target of the E5 protein in these cells as well as C127 cells. Here, we assessed the ability of the E5 protein to form a complex with the EGF receptor as well as with the PDGF receptor in NMuMG cells. As assessed by PDGF receptor Western blotting (Fig. 3A), the PDGF receptor precursor is present in E5 immunoprecipitates from extracts of cells expressing PDGF β receptor and the E5 protein, but not from cells expressing PDGF receptor alone, as previ-

ously reported (31). In contrast, E5 immunoprecipitates contain no detectable EGF receptor, as assessed by anti-EGF receptor immunoblotting (Fig. 3B). Therefore, in NMuMG epithelial cells expressing the E5 protein and the PDGF receptor as well as abundant endogenous EGF receptor, the E5 protein specifically interacts with and activates the PDGF receptor. Even in the absence of PDGF receptors, the E5 protein does not interact with endogenous EGF receptors in NMuMG cells (data not shown). Therefore, in a variety of stably transformed cell lines, the E5 protein specifically forms a complex with the PDGF β receptor and induces its activation. In contrast, the E5 protein does not form a complex with the EGF receptor in these cells, nor does E5 expression result in EGF receptor activation.

Target specificity of the E5 protein in COS cells. The ability of the E5 protein to interact with a panel of growth factor receptors was examined in monkey COS cells. COS cells transiently overexpressing the E5 protein and either the human PDGF β receptor, the human receptor for CSF-1, the rat *neu* oncogene product, p185^{neu}, or the human EGF receptor were subjected to coimmunoprecipitation analysis. First, antireceptor immunoprecipitates from extracts of metabolically labeled cells were analyzed for the presence of the E5 protein by gel electrophoresis (Fig. 4). The E5 protein coimmunoprecipitates not only with the human PDGF β receptor (lane 3) but also with the CSF-1 receptor (lane 7), p185^{neu} (lane 11), and the EGF receptor (lane 15). Each receptor antiserum immunoprecipitated readily detectable amounts of the appropriate receptor as well (data not shown). The presence of a higher amount of the E5 protein in CSF-1 receptor immune complexes (lane 7) than in other receptor immune complexes most likely reflects the higher level of CSF-1 receptor expression attained in this experiment (data not shown). The E5 protein was not present in receptor immunoprecipitates from extracts of cells expressing the E5 protein in the absence of the receptors (lanes 2, 6, 10, and 14), indicating that the antireceptor antibodies did not cross react with the E5 protein. Thus, in

FIG. 4. Coimmunoprecipitation of the E5 protein with various receptors from COS cell extracts by using antireceptor antisera. COS cells were transfected with 0.5 μ g of pMT2E5 (which contains the E5 gene) and of one of the following receptor genes as indicated: 12 μ g of pPRCHH-8 (PDGF β receptor [PDGF R]), 0.5 μ g of pSR α -CSF-1R (CSF-1 receptor [CSF-1 R]), 1 μ g of pSR α -neuN (p185^{neu}), or 1 μ g of pSR α -EGFR (EGF receptor [EGF R]). Metabolically labeled extracts were immunoprecipitated as indicated with either a nonimmune rabbit serum (NI) or one of the following receptor antibodies: PR4 (P), anti-CSF-1 receptor antiserum 4069 (C), p185^{neu} monoclonal antibody Ab4 (n), or EGF receptor monoclonal antibody LA22 (E). Immunoprecipitates were electrophoresed on an SDS-10 to 20% polyacrylamide gel. Lanes 1 to 3, 5 to 7, 9 to 11, and 13 to 15 represent equal amounts of incorporated counts immunoprecipitated with each receptor antiserum. Lanes 4, 8, 12, 16, and 17 were immunoprecipitated with the E5 antiserum and represent 20% of the incorporated counts in the other lanes. The arrow on the right indicates the position of the E5 protein. Sizes in kilodaltons are indicated on the left.

FIG. 5. Coimmunoprecipitation of various growth factor receptors with the E5 protein from COS cell extracts by using an E5 antiserum. COS cells were either mock transfected or transfected with 20 μ g of pPRCHH-8 (which expresses PDGF β receptor [PR]; A), 30 μ g of pSV7d-CSFR (which expresses CSF-1 receptor [CR]; B), 30 μ g of pSR α -neuN (which expresses p185^{neu} [p185]; C), or 30 μ g of pSR α -G (which expresses VSV-G; D) in the presence (+) or absence (-) of 1 μ g (A to C) or 10 μ g (D) of pMT2E5 (which expresses the E5 protein), as indicated. Cell extracts were subjected to coimmunoprecipitation with the α -E5 antiserum followed by Western blot analysis with either anti-PDGF receptor PR4 (A), anti-CSF-1 receptor 4069 (B), anti-p185^{neu} Ab3 (C), or anti-VSV (D) antiserum. The left half of each panel shows the total amount of protein expressed, as documented by immunoprecipitation with the respective antireceptor antiserum followed by immunoblotting with the same antiserum; lane 5 of panels A, C, and D and lane 4 of panel B show the amount of receptor coimmunoprecipitated by the E5 antiserum. In panel B, CSF-1 receptor was affinity purified from extracts or α -E5 immune complexes by using WGA as described in Materials and Methods. The amounts of extracted protein used per lane were as follows: (A) 133 μ g (lanes 1 to 3) and 1,380 μ g (lanes 4 and 5); (B) 150 μ g (lanes 1 and 2) and 1,500 μ g (lanes 3 and 4); (C) 100 μ g (lanes 1 to 3) and 450 μ g (lanes 4 and 5); (D) 124 μ g (lanes 1 to 3) and 372 μ g (lanes 4 and 5). Arrowheads on the right indicate positions of mature and precursor forms of either the PDGF receptor (PR; A) or the CSF-1 receptor (CR; B) or the position of p185^{neu} (p185; C) or VSV-G (D). Sizes in kilodaltons are indicated on the left.

COS cells, the E5 protein associates not only with the PDGF receptor but also with the CSF-1 receptor, p185^{neu}, and the EGF receptor. It is important to note that the E5 protein does not coimmunoprecipitate with endogenous p185^{neu} and EGF receptor in COS cells (lanes 10 and 14, respectively, and data not shown). COS cells do not express detectable levels of endogenous PDGF receptor or CSF-1 receptor.

Immunoprecipitation with the anti-E5 antiserum followed by immunoblotting with antibodies against the appropriate receptor further demonstrated that the E5 protein can interact with a variety of receptors in COS cells (Fig. 5). The human PDGF β receptor (Fig. 5A), the CSF-1 receptor (Fig. 5B), and p185^{neu} (Fig. 5C) can be detected in E5 immune complexes from extracts of cells expressing the E5 protein and each receptor. In the case of the structurally related PDGF and CSF-1 receptors, the E5 protein specifically associates with the

immature receptor forms. The E5 antiserum did not cross-react with any of the receptors, since it did not precipitate receptor from extracts of cells expressing receptor in the absence of the E5 protein. Similarly, as shown in Fig. 6B, human EGF receptor is present in E5 immunoprecipitates from COS cell extracts. These results indicate that under conditions of transient overexpression in COS cells, the E5 protein interacts with a variety of growth factor receptors. In contrast, the E5 protein and the transmembrane glycoprotein of VSV (VSV-G) could not be coimmunoprecipitated from extracts of COS cells coexpressing these two proteins (Fig. 5D), indicating that the E5 protein does not form a complex with VSV-G in COS cells. The E5 protein may selectively interact with growth factor receptor-type proteins in COS cells. On the other hand, it is possible that the location of VSV-G within the cell or some other feature of VSV-G prevents an

FIG. 6. Competition between mouse PDGF β receptors and human EGF receptors for complex formation with the E5 protein in COS cells. COS cells were transfected with 30 μ g of pSVR1 (PDGF receptor [PR]), 7.5 μ g of pSR α -EGFR (EGF receptor [ER]), and 1 μ g of pMT2E5 (E5), as indicated below each panel. Unlabeled (A and B) or metabolically labeled (C) cell extracts were immunoprecipitated with anti-PDGF receptor PR4 (α PR [P]), anti-EGF receptor 06-129 (α -ER [E]), or anti-E5 (α E5) antiserum or a nonimmune rabbit serum (N), as indicated above each panel. Immunoprecipitates were electrophoresed, and gels were subjected either to immunoblot analysis using α -PR4 (A) or α -ER (B) or to fluorography followed by autoradiography (C). In panels A and B, lanes 1 to 3 represent 100 (A) or 50 (B) μ g of extracted protein, while lanes 5 and 6 represent 585 μ g of extracted protein. In panel C, equal amounts of incorporated counts were used for each lane. Arrowheads on the right indicate the positions of the precursor form of the PDGF receptor (PR) in panel A, the EGF receptor (ER) in panel B, and the mature and precursor PDGF receptor forms in panel C. The arrowhead on the left in panel C indicates the position of the EGF receptor. Sizes in kilodaltons are indicated on the left.

interaction with the E5 protein. Nonetheless, there appears to be little specificity in the binding of the E5 protein to growth factor receptor tyrosine kinases in COS cells, whereas in stably transformed cells, the E5 protein specifically interacts with the PDGF β receptor.

To explore this difference between stable transformants and the COS cell system, we assessed the ability of the PDGF β receptor to compete with the EGF receptor for binding to the E5 protein in COS cells. The ability of the E5 protein to form a complex with one receptor was assessed in the presence or absence of the other receptor. COS cells were transfected with various combinations of E5, mouse PDGF β receptor, and human EGF receptor genes. Extracts were subjected to coimmunoprecipitation with the E5 antiserum, and the presence of EGF receptors or PDGF β receptors in E5 immunoprecipitates was determined by immunoblotting with the appropriate antireceptor antiserum (Fig. 6). As noted above, the E5 protein can form a complex with either the mouse PDGF β receptor or the human EGF receptor when each receptor is expressed in the absence of the other receptor in COS cells (Fig. 6A and B, lanes 5). An extract was also prepared from cells transfected with both the EGF receptor and the PDGF receptor cDNAs together with the E5 gene. After immunoprecipitation with the E5 antiserum, the E5 immune complex was divided in two and analyzed for the presence of both types of receptor. As shown in Fig. 6A, lane 6, coexpression of the EGF receptor had little effect on the amount of PDGF receptor present in the E5 immune complex. In contrast, as shown in Fig. 6B, lane 6, coexpression of the PDGF receptor caused a dramatic reduction in the amount of EGF receptor in a complex with the E5 protein. Coexpression of the PDGF receptor did not significantly affect EGF receptor expression, demonstrating that the reduction in the amount of EGF receptor in a complex with the E5 protein was not caused by reduced EGF receptor expression in the cotransfected cells (Fig. 6B [compare lanes 2 and 3]; 6C [compare lanes 4 and 6]). After correction for the slight variations in the amounts of

receptors expressed in cells transfected with one receptor or both receptor genes, PDGF receptor coexpression causes approximately an 8-fold reduction in the amount of EGF receptor in a complex with the E5 protein, whereas the presence of EGF receptor reduces the amount of PDGF receptor in the complex less than 1.3-fold. Similar results were obtained in four additional independent transfection experiments.

To assess the absolute levels of EGF receptors and PDGF receptors in cotransfected COS cells, cells transfected in parallel with those analyzed in Fig. 6A and B were metabolically labeled with [35 S]cysteine. Labeled extracts were subjected to immunoprecipitation with saturating amounts of antireceptor antisera followed by gel electrophoresis and receptor quantitation using phosphorimage analysis after correction for cysteine content (Fig. 6C). Cotransfected cells express approximately eight times more EGF receptor than PDGF receptor. Thus, the presence of a relatively small amount of PDGF receptor can effectively compete with an excess of EGF receptor for binding to the E5 protein in COS cells, indicating that the E5 protein preferentially interacts with the PDGF β receptor in COS cells as it does in stably transformed mouse cells and bovine fibroblasts.

DISCUSSION

The E5 protein does not indiscriminately bind and activate growth factor receptors in stable transformants, but rather it displays considerable specificity towards these cellular proteins. In the experiments presented here, the E5 protein specifically interacts with the PDGF β receptor and not the EGF receptor. We examined three independently derived pairs of stable cell lines: bovine fibroblasts which express endogenous PDGF receptors and EGF receptors, mouse C127 cells which express endogenous PDGF receptors and exogenous human EGF receptors, and mouse NMuMG cells which express exogenous mouse PDGF receptors and endogenous

EGF receptors. In all three cases, a clear increase in tyrosine phosphorylation of the PDGF receptor was observed in response to BPV E5 expression, whereas we were unable to detect E5-induced tyrosine phosphorylation of the EGF receptor in the same cells. We also detected tyrosine phosphorylation of the PDGF receptor and not the EGF receptor in response to E5 expression in a fourth cell line, rat FR 3T3 fibroblasts (32, 34). In addition, in all three stably transformed cell lines, the E5 protein specifically forms a complex with the PDGF receptor and not the EGF receptor. In bovine fibroblasts and mouse C127 cells, phosphotyrosine immunoblotting of E5 immunoprecipitates failed to detect E5-associated tyrosine-phosphorylated proteins other than PDGF β receptor, indicating that it is the major if not the only growth factor receptor bound and activated by the E5 protein. In the bovine cells, the PDGF β receptor is preferentially activated in comparison with the closely related PDGF α receptor. The ability of the E5 protein to discriminate between the PDGF α and the PDGF β receptor has also been observed by Goldstein et al. (17). Furthermore, when the mouse PDGF β receptor and the human EGF receptor are coexpressed in COS cells, the E5 protein preferentially interacts with the PDGF receptor in comparison with the EGF receptor. Therefore, when presented with EGF receptors and PDGF β receptors at various expression levels, the E5 protein consistently associates with and activates the PDGF β receptor and not the EGF receptor.

This identification of the PDGF β receptor as the preferred target of the E5 protein is consistent with our earlier finding that the PDGF β receptor but not the EGF receptor can mediate a transforming signal from the E5 protein in NMuMG cells (31). Moreover, a variant C127 cell line expressing reduced levels of PDGF receptors displays an impaired mitogenic response to PDGF treatment and to E5 expression but a normal response to EGF, suggesting that an intact PDGF receptor signaling pathway is required for efficient E5 function in these mouse fibroblasts (37). Finally, Goldstein et al. recently found that in the murine myeloid cell line 32D, the PDGF β receptor but not the EGF receptor is activated by the E5 protein, and that only the PDGF β receptor can transmit a proliferative signal in this system (17). Thus, in six cell lines (bovine fibroblasts, mouse C127, NMuMG, 32D, rat FR 3T3, and monkey COS cells) from various species and of various lineages, the E5 protein interacts preferentially with the PDGF β receptor.

The E5 protein selectively forms a complex with and activates the PDGF β receptor in all of the transformed cell lines examined here, providing further evidence that activation of the PDGF receptor by the E5 protein requires a physical association between these two protein (8, 33). If each E5 monomer binds a single PDGF receptor molecule, then the dimeric nature of the E5 protein would cause receptor dimerization, which in turn may result in activation. Although complex formation between the E5 protein and the PDGF receptor appears to be required for receptor activation, there is evidence that it is not sufficient. Goldstein et al. have reported that the entire carboxyl-terminal domain of the E5 protein can be deleted without impairing complex formation with the PDGF receptor in COS cells (14), but genetic and peptide injection experiments indicate that carboxy-terminal amino acids are critical for E5 biological activity in C127 cells (19, 21, 22, 36, 46). Systematic analysis of E5 mutants in fibroblasts should clarify the role of the various domains of the E5 protein in PDGF receptor association and activation.

In contrast to complex formation between the E5 protein and the PDGF β receptor which has been detected in numerous stably transformed cell types, including bovine fibroblasts

expressing endogenous PDGF receptor, a physical interaction between the E5 protein and the EGF receptor has been demonstrated only for exogenous EGF receptors in COS cells (8). Our results confirm that the E5 protein can interact with several growth factor receptors in COS cells, but it is important to question the physiological significance of interactions which occur under conditions of protein overexpression in COS cells. Protein overexpression is likely to result in a high local concentration of transmembrane proteins in cell membranes, thereby facilitating the formation of complexes or nonspecific aggregates which might otherwise not occur (29). For example, the CSF-1 receptor and the BPV E5 protein can form a complex in COS cells, but this receptor is not expressed in the natural host cells of BPV. Moreover, overexpression of transfected receptors may explain why the E5 protein interacts with transfected but not endogenous EGF receptors in COS cells. However, some specificity exists in the COS cell system because the preference of the E5 protein for the PDGF β receptor compared with the EGF receptor is retained as shown in cells coexpressing both receptors, and no complex formation occurs between the E5 protein and the VSV-G protein. Therefore, although protein overexpression may allow the detection of some interactions that are not physiologically relevant, our results suggest that the E5 protein has the potential to undergo specific interactions with several growth factor receptors in addition to the PDGF β receptor.

The mechanism by which the E5 protein interacts with several different growth factor receptors is not clear. Cohen et al. (8) have presented data that the E5 protein interacts with the transmembrane domain of the PDGF receptor and the cytoplasmic domain of the EGF receptor, resulting in receptor activation in both cases. The receptor domains required for binding to the E5 protein were determined in studies of chimeric receptors overexpressed in COS cells. Given the complications of the COS cell assay system noted above, the ability of the E5 protein to recognize different specific receptor domains warrants examination under more natural conditions and expression levels. It is also possible that instead of (or in addition to) directly binding to growth factor receptors, the E5 protein associates with another protein which in turn mediates binding to several growth factor receptors. An α -adaptin-like protein (9) and the 16-kDa subunit of a vacuolar H^+ -ATPase (14, 15, 18) have been shown to associate with the E5 protein and thus have been speculated to serve as such adaptor-like proteins. However, the functional significance of these interactions is unclear because transformation-defective E5 mutants can interact with these proteins, some transformation-competent mutants are defective for complex formation with the 16-kDa protein, and expression of the E5 protein has not been shown to influence the activity of these cellular proteins (9, 16, 25).

Our observations are in contrast to those of Cohen et al., who reported that E5 expression in a different cell line, murine NIH 3T3 cells, results in activation of exogenous human EGF receptors as well as of the endogenous PDGF receptors (8). Furthermore, Martin et al. observed that the steady-state level of exogenous EGF receptor in NIH 3T3 cells was increased in response to E5 expression (30). In the case of our C127 cell lines expressing human EGF receptor, we did not observe a consistent difference in the steady-state level of EGF receptors in the presence or absence of E5 expression. The lack of complex formation between the E5 protein and the EGF receptor in the C127 cells may account for the absence of an obvious effect of the E5 protein on EGF receptor metabolism in our experiments. We do not understand why the E5 protein is able to influence the activity of the EGF receptor in NIH

3T3 cells but not in C127, FR 3T3, NMuMG, and 32D cells or in bovine fibroblasts. It is possible that a cofactor required for this interaction is expressed by NIH 3T3 cells but not by the cell types studied here. If this is the case, then a cell's response to the E5 protein would be determined both by the expression of growth factor receptors and by the expression of other proteins that influence the interaction of the E5 protein with these receptors. Therefore, our results do not rule out the possibility that the EGF receptor is a target of the E5 protein in certain cell types.

We conclude that the PDGF β receptor is the major cellular target for the E5 protein in a variety of cell types, including bovine fibroblasts, and that the interaction between the E5 protein and the PDGF receptor results in receptor activation which in turn delivers a sustained proliferative signal to the cell. Activation of this pathway not only results in transformation of cells in vitro but also is likely to play a major role in the fibrotropism of the fibropapillomaviruses in vivo, since dermal fibroblasts and other mesenchymal targets of BPV tumorigenesis typically express abundant PDGF receptor. In addition, the results obtained in COS cells indicate that the BPV E5 protein has the potential to interact with a variety of other growth factor receptors. Such interactions may be important for the biological response of other cell types to BPV. In vivo, BPV induces the proliferation of keratinocytes as well as fibroblasts. The BPV E5 protein is expressed in basal layer keratinocytes in bovine fibropapillomas, and the E5 protein transforms cultured epidermal keratinocytes as well as fibroblasts (5, 28). Since keratinocytes do not express PDGF β receptors, the E5 protein must recognize a different target in these cells. Evidently, this target or its downstream substrates is not present or is inaccessible in NMuMG and 32D cells, since E5-mediated transformation of these cells occurs only if the PDGF β receptor is expressed (17, 31). We can speculate that the E5 transforming proteins of the human papillomaviruses, which in humans induce proliferation of keratinocytes exclusively, interact with a keratinocyte-specific target which may be identical to the putative keratinocyte-specific target recognized by the BPV E5 protein. Our observation that the BPV E5 protein can interact with a variety of growth factor receptors in COS cells, as well as biochemical and physiological data obtained by others (8, 27, 35, 47), suggest that the putative keratinocyte-specific targets of papillomavirus E5 proteins may also be growth factor receptors.

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