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Are transforming properties of the bovine papillomavirus E5 protein shared by E5 from high-risk human papillomavirus type 16?

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Abstract

The E5 proteins of bovine papillomavirus type 1 (BPV-1) and human papillomavirus type 16 (HPV-16) are small (44–83 amino acids), hydrophobic polypeptides that localize to membranes of the Golgi apparatus and endoplasmic reticulum, respectively. While the oncogenic properties of BPV-1 E5 have been characterized in detail, less is known about HPV-16 E5 due to its low expression in mammalian cells. Using codon-optimized HPV-16 E5 DNA, we have generated stable fibroblast cell lines that express equivalent levels of epitope-tagged BPV-1 and HPV-16 E5 proteins. In contrast to BPV-1 E5, HPV-16 E5 does not activate growth factor receptors, phosphoinositide 3-kinase or c-Src, and fails to induce focus formation, although it does promote anchorage-independent growth in soft agar. These variant activities are apparently unrelated to differences in intracellular localization of the E5 proteins since retargeting HPV-16 E5 to the Golgi apparatus does not induce focus formation.

Keywords: E5 protein; Bovine papillomavirus type 1; Human papillomavirus type 16; Golgi apparatus

Introduction

Bovine papillomavirus type 1 (BPV-1) is a double-stranded DNA tumor virus that induces benign neoplasia in cattle (Lancaster and Olson, 1982) and horses (Carr et al., 2001), and has been used as a model system to study papillomavirus-mediated transformation in vitro (Chen et al., 1982; Dvoretzky et al., 1980; Lowy et al., 1980). The major in vitro transforming protein of BPV-1 is E5 (BPV E5) (DiMaio et al., 1986; Groff and Lancaster, 1986; Schiller et al., 1986; Yang et al., 1985), a small (44 amino acids), hydrophobic polypeptide that localizes to membranes of the Golgi apparatus (Burkhardt et al., 1989; Schapiro et al., 2000). The transforming activity of BPV E5 derives in part from its ability to induce trans-phosphorylation and constitutive activation of platelet-derived growth factor receptors (PDGF-Rs) that form a complex with BPV E5 dimers (Drummond-Barbosa et al., 1995; Goldstein et al., 1994; Lai et al., 1998; Petti et al., 1991). In addition, BPV E5 binds to the 16-kDa pore-forming subunit of the vacuolar H^+-ATPase (16K) (Goldstein et al., 1991) and inhibits normal acidification of the Golgi lumen by interfering with H^+ transport (Schapiro et al., 2000). The interaction of BPV E5 with 16K may contribute to E5-mediated transformation, since the Q17S and L26A BPV E5 point mutants inhibit Golgi acidification and transform fibroblasts (Schapiro et al., 2000), but do not activate the PDGF-R because they are defective in PDGF-R binding or homodimer formation, respectively (Adduci and Schlegel, 1999; Sparkowski et al., 1996). These E5 mutants activate heterodimeric phosphoinositide 3-kinase (PI 3-K) and c-Src in a PDGF-R-independent manner, and c-Src activation is required for transformation by L26A E5 (Suprynowicz et al., 2000, 2002).

Epidemiological studies have shown that high-risk human papillomaviruses (HPVs) are the primary causative agent of cervical cancer worldwide, and that HPV E5, E6 and E7 genes play important roles in HPV pathogenesis (zur Hausen,

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2002). While the E6 and E7 oncogenes of high-risk HPV type 16 (HPV-16) have been studied extensively, considerably less is known about the biological activity of its E5 gene product (HPV-16 E5). Like BPV E5, HPV-16 E5 is a small (83 amino acids), hydrophobic protein that localizes to intracellular membranes (Conrad et al., 1993; Disbrow et al., 2003), and can transform fibroblasts to grow in soft agar (Leechanachai et al., 1992; Pim et al., 1992; Straight et al., 1993). HPV-16 E5 also is mitogenic in primary keratinocytes, especially in cooperation with epidermal growth factor (EGF) and HPV-16 E7 (Bouvard et al., 1994; Straight et al., 1993; Valle and Banks, 1995; Venuti et al., 1998). The latter finding may be related to the ability of HPV-16 E5 to enhance HPV-16-mediated reprogramming of terminally differentiating keratinocytes to undergo DNA synthesis in order to support viral DNA amplification (Genther et al., 2003). HPV-16 E5 stably associates with the 16K V-ATPase protein (Adam et al., 2000; Ashby et al., 2001; Conrad et al., 1993; Rodriguez et al., 2000), and can bind to the EGF receptor (EGF-R), PDGF-R and colony-stimulating factor-1 receptor (Hwang et al., 1995). The association of wild-type (wt) HPV-16 E5 with 16K may interfere with vacuolar H^+-ATPase function and inhibit endosome acidification (Adam et al., 2000; Briggs et al., 2001; Straight et al., 1995), although an inhibitory effect on endocytic trafficking also appears likely (Ashby et al., 2001; Thomsen et al., 2000). The resulting delay in endosome acidification (Straight et al., 1993, 1995), or delay in the fusion of early endocytic vesicles with acidic late endosomes (Rodriguez et al., 2000; Thomsen et al., 2000), may be responsible for enhanced EGF-R activation by EGF in cells expressing HPV-16 E5 (Crusius et al., 1998; Pim et al., 1992; Rodriguez et al., 2000; Straight et al., 1993).

The inability of previous studies to detect expression of the HPV-16 E5 protein, unless transiently overexpressed in COS cells (Conrad et al., 1993; Hwang et al., 1995), undoubtedly has hampered characterization of its biological activity, and may account for discrepancies in its reported properties. Recently, Disbrow et al. (2003) have shown that HPV-16 E5 is expressed poorly in mammalian cells due to a high incidence (40%) of infrequently used codons, and that expression can be increased greatly by substituting codons more commonly used to encode the same amino acids. In the present study, we use codon-optimized HPV-16 E5 DNA to generate fibroblast cell lines that stably express epitope-tagged HPV-16 E5 and BPV E5 proteins at the same level. This system enables us to directly determine whether HPV-16 E5 exhibits oncogenic properties of BPV E5 in cells where the behavior of BPV E5 is well characterized.

Results

Growth factor receptor activation

To investigate whether HPV-16 E5 activates signal transduction proteins known to be activated by BPV E5, we generated NIH 3T3 cell lines that stably express the E5 proteins in equal amounts. NIH 3T3 cells were chosen for this purpose since the signaling properties of BPV E5 have been characterized extensively in these cells (Goldstein et al., 1994; Lai et al., 1998; Petti et al., 1991; Sparkowski et al., 1996; Suprynowicz et al., 2000, 2002). Normally, the HPV-16 E5 gene is expressed poorly in mammalian cells due to a high incidence (40%) of infrequently used codons, but expression can be increased more than sixfold by replacing these codons with more commonly used counterparts (Disbrow et al., 2003). As shown in Fig. 1, codon-optimized HPV-16 E5 and wt (not codon-optimized) BPV E5 proteins were present at equivalent levels when epitope-tagged and expressed in 3T3 cell lines. No E5 expression was detected in a control cell line harboring the empty JS55 expression vector. The immunoprecipitation and immunoblotting protocol used to detect E5 was not saturated by the amount of cell lysate employed in these measurements (1.5 mg), since identical results were obtained when E5 proteins were immunoprecipitated from half as much lysate (0.75 mg; Fig. 1). E5 expression remained stable in these cell lines for at least 30 passages (data not shown).

The ability of BPV E5 to induce tyrosine phosphorylation and sustained activation of the PDGF-R is well documented (Drummond-Barbosa et al., 1995; Goldstein et al., 1994; Lai et al., 1998; Petti et al., 1991). In our BPV E5-expressing cell line, the PDGF-R (immature form) was constitutively activated, especially after 24 h of serum starvation. In contrast, persistent activation of the PDGF-R was not observed in cell lines expressing HPV-16 E5 or containing the empty JS55 expression vector (Fig. 2A). Serum-starved HPV-16 E5-expressing cells and vector control cells exhibited equally substantial activation of the PDGF-R (mature form) when acutely stimulated with PDGF for 10 min (Fig. 2A), thereby demonstrating that HPV-16 E5 did not inhibit receptor activation. It is noteworthy that HPV-16 E5 also did not enhance PDGF-R activation in the presence of PDGF (Fig. 2A), given its ability to increase the recycling of ligand-bound EGF-Rs to the plasma membrane in keratinocytes (Straight et al., 1993, 1995).

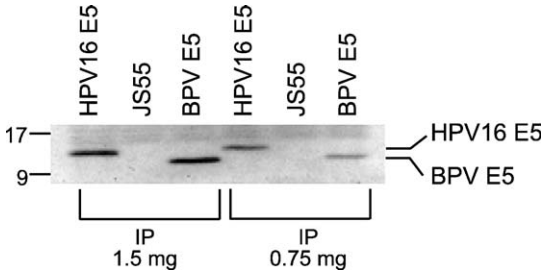

Fig. 1. Expression levels of BPV E5 and HPV-16 E5 in NIH 3T3 cell lines. Anti-AU1 Western blot of anti-AU1 immunoprecipitates from stable cell lines expressing AU1-tagged HPV-16 E5, BPV E5 or harboring the empty JS55 expression vector. Immunoprecipitates were prepared from 1.5 or 0.75 mg of cell lysate protein. Molecular mass markers (in kDa) are indicated on the left.