Allosteric Activation of Acid α-Glucosidase by the Human Papillomavirus E7 Protein*

(Received for publication, November 23, 1999)

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Changes in the cellular carbohydrate metabolism are a hallmark of malignant transformation and represent one of the earliest discernible events in tumorigenesis. In the early stages of certain epithelial cancers, a metabolic switch is regularly observed, in which slowly growing glycogenetic cells are converted to highly proliferating basophilic cells. This step is accompanied by a rapid depletion of the intracellular glycogen stores, which in liver carcinogenesis results from the activation of the enzyme acid α-glucosidase by an as yet unknown mechanism. We show here that acid α-glucosidase is a target for the E7 protein encoded by human papillomavirus type 16, a human tumor virus that plays a key role in the genesis of cervical carcinoma. We show that expression of E7 induces the catalytic activity of acid α-glucosidase in vivo and wild type E7, but not transformation-deficient mutants bind directly to acid α-glucosidase and increase the catalytic activity of the enzyme in vitro. The data suggest that the E7 protein encoded by human papillomavirus type 16 can act as an allosteric activator of acid α-glucosidase.

It has long been known that tumor cells display characteristic alterations of the carbohydrate metabolism (for review see Ref. 1), which represent one of the earliest discernible events in tumorigenesis (for recent review see Ref. 2). In certain epithelial cancers, such as liver cancer or kidney cancer, one of the first detectable alterations is a metabolic switch, in which slowly growing glycogenetic cells, also referred to as clear cells, are converted to highly proliferating basophilic cells (for review see Ref. 3). This step is accompanied by a rapid depletion of the intracellular glycogen stores, which in liver carcinogenesis results from the activation of acid α-glucosidase (4), whereas the activity of glycogen phosphorylase, the other cellular glycogen-degrading enzyme, is reduced throughout hepatocarcinogenesis (5). The actual content of glycogen in a cell is controlled through the balance of glycogen-synthesizing enzymes and glycogen-degrading enzymes, which themselves are under allosteric control by various metabolites (6). It is unknown at present how the activity of these enzymes is modulated in early carcinogenesis, to first build up the clear cell phenotype and then trigger its disappearance at later stages. According to the current concept (7), the deregulation of metabolic enzymes in early tumor cells reflects changes in cellular signal transduction which lead to tumor-specific alterations of the metabolic apparatus.

Reduced glycogen storage is regularly observed in early lesions of the cervix, a finding that has been used for clinical diagnosis of cervical dysplasia for more than 60 years (8). Cervical neoplasia is tightly linked to infection by human papillomaviruses (HPV) of the high risk group, e.g. HPV-16 (9), and two viral genes, E6 and E7, are required for papillomavirus-associated carcinogenesis (for review see Refs. 9 and 10). The E7 oncogene of HPV-16 cooperates with HPV-16 E6 to immortalize human keratinocytes (11). The transforming activity of E7 is sensitive to mutations in the N-terminal domain (12, 13), and it was shown that the N-terminal part of the HPV-16 E7 oncoprotein mediates binding to proteins of the retinoblastoma gene family (14). Therefore E7 triggers activation of cellular genes driven by the E2F transcription factor (15), leading to the accumulation of cellular factors required for S phase entry (for review see Refs. 16 and 17).

However, the ability of E7 to transform cells is also sensitive to mutations in the C-terminal domain (18), which is of particular importance for the ability of E7 to immortalize human keratinocytes, the natural host cells of HPV-16 (19). Furthermore, whereas it was convincingly demonstrated that the interaction of E7 with nuclear proteins is essential for its transforming potential (reviewed in Ref. 20), a substantial fraction of the total E7 protein is found in the cytoplasm (21, 22). In accordance with these observations, it was found that several cellular proteins interact with the C terminus of HPV-16 E7 (23–28), and one of these additional E7 target proteins, the glycolytic control enzyme M2 pyruvate kinase (M2-PK) (28), is localized in the cytoplasm. M2-PK plays a key role in reprogramming the cellular carbohydrate metabolism in tumors (for review see Ref. 29), and it was shown that HPV-16 E7 shifts M2-PK to the tumor-specific dimeric form with decreased substrate affinity, resulting in the expansion of the intracellular pools of glycolytic phosphometabolites (28).

In an attempt to discover additional E7-binding proteins, we now identified the glycogen-degrading enzyme acid α-glucosi-

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* This work was supported by grants from the Deutsche Forschungsgemeinschaft, the European Union (Biomed 2 program), and the Deutsche Krebshilfe. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1. The abbreviations used are: HPV, human papillomaviruses; Dex, dexamethasone; GST, glutathione S-transferase; DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine isothiocyanate.

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Fig. 1. Identification of acid α-glucosidase as E7-binding protein. A, a plasmid pLexA-16 E7(39-98)::HIS3 encodes the DNA binding domain of LexA fused in frame to the C-terminal part of HPV-16 E7 and the HIS3 selectable marker. The structure of the expression library, derived from vector pJG4-5 by insertion of cDNAs from a WI-38 cDNA library, is indicated. B42-TAD refers to the B42 transactivation domain. B42 fusion proteins are expressed from the inducible GAL1 promoter, and these plasmids contain the TRP1 gene as selectable marker. B, the plasmid designated pB42-acid-α-Gluc::TRP1 was isolated during the interaction screen; it contains the cDNA for human acid α-glucosidase (37). C, derivatives of yeast strain EGY48/pSH1834, expressing various LexA fusion proteins as indicated, were transformed with the plasmid pB42-acid-α-Gluc::TRP1. pJG4-5, expressing the unfused B42 trans-activation domain, was used as negative control. Transformants were selected for uracil, histidine, and tryptophan prototrophy and grown in glucose minimal medium (Ura-, His+, and Trp+) Yeast cells were then streaked out onto each of three plates and incubated for 4 days at 30 °C under the following nutrient conditions: control, glucose, glucose minimal medium without leucine, selection for B42 fusion protein-independent activation of the LexA::LEU2 reporter; galactose, galactose minimal medium with leucine, selecting for B42 fusion protein dependent activation of the LexAo6-LEU2 gene.

**EXPERIMENTAL PROCEDURES**

**Interaction Analysis in Yeast**—The C-terminal part of HPV-16 E7, fused in frame to the DNA-binding domain of LexA, was used as a bait in the yeast two-hybrid system (30), to identify cDNAs for E7-binding proteins from a human cDNA expression library (31). Yeast strain EGY48/pSH1834 (Mat a, his3, ura3, trp1, leu2::lexAo6-pLEU2/lexAo8-Gal1-lacZ::URA3) (28) was used for both the LEU2 and β-galactosidase reporter gene assays. For determination of reporter gene activity, EGY48/pSH1834 was transformed with plasmids expressing various LexA fusion proteins, together with the plasmid pB42-acid-α-Gluc::TRP1, and selected for leucine prototrophy as described (32); alternatively, β-galactosidase activity was determined in cellular extracts as described (32). The expression vector for LexA-16E7Δ79-83 was constructed as follows. A polylinker (oligonucleotide I, 5′-AATTC-CATGGGGATCCCGGGGTCGACGGATCCC3′) was inserted into the EcoRI and NcoI sites of pEG202 (31), generating the plasmid pEG202-BamHI linker. The 16E7Δ79-83 cDNA was isolated from pJ4Δ1tal-79-83 (27) as BamHI, EcoRI (repair) fragment and inserted into the plasmid pEG202-BamHI linker treated with BamHI and Ncol (repair). Construction of the other LexA fusion protein expression vectors was described (30). All LexA fusion proteins were expressed to the same level, as confirmed by direct immunoblotting, using a polyclonal antibody to LexA (30).

In Vitro Interaction Analysis—GST fusion proteins containing various mutants of E7 were loaded (20 ng/μl each) on glutathione-Sepharose 4B beads. Yeast lysates (1 mg) or purified mature and premature acid α-glucosidase proteins (200 pg/μl) were incubated with GST-E7 fusion proteins, and bound proteins were analyzed by Western blotting (28), using anti-α-glucosidase antibodies (33). Input of the various GST-derived fusion proteins was controlled by Coomassie staining. Levels of acid α-glucosidase proteins bound to GST-E7 were quantitated by densitometric scanning of the autoradiogram.

**Cell Lines**—The cell line 14/Δ79-83 was generated from primary baby rat kidney cells by stable expression of an activated ras oncogene, combined with glucocorticoid-inducible expression of the HPV-16 E7 mutant Δ79-83 (27), as described before for 14/2 cells (34). Colonies were selected from the transfection and analyzed for E7 gene expression by Western blot. Normal rat kidney cells were obtained from ATCC (NRK-49F, Batch F-13632). Cells were cultured in DMEM + 10% FCS and dexamethasone, as described previously (28). U2-OS cells, a human osteosarcoma cell line (35), were cultured in DMEM + 10% FCS.

**Cell Fractionation Experiments**—For subcellular fractionation, a method according to Schuelke et al. (36) was used. Cells were pelleted by centrifugation and lysed for 5 min at 0 °C in two packed cell volumes of hypotonic lysis buffer (10 mM HEPES-KOH, pH 7.5, 10 mM KCl, 3 mM MgCl2, 1 mM EDTA, 0, 2 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 5 mg/ml aprotinin, 1 mM NaF, 0, 1 mM NaVO3). After addition of Nonidet P-40 to a final concentration of 0.05% (v/v) and incubation for 5 min at 0 °C, nuclei were pelleted by centrifugation at 1000 × g, washed twice in hypotonic lysis buffer, and extracted in high salt extraction buffer (10 mM HEPES-KOH, pH 7.5, 500 mM KCl, 5 mM MgCl2, 0.5 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 5 mg/ml aprotinin, 1 mM NaF, 0.1 mM NaVO3). Protein extracts from the cytoplasmic (1000 × g supernatant) and nuclear fraction were subjected to immunoblotting using antibodies against lamin B (antibody-1, Calbiochem), M2-PK (DF4, ScheBoTech, Wettenberg, Germany), calreticulin (a gift from H. D. Sölting), and HPV16 E7 (a gift from J. Bransenpen).

**Indirect Immunofluorescence**—U2-OS cells were cultured in DMEM + 10% FCS. For transient expression of cDNAs, cells were grown to about 80% confluence on glass coverslips coated with 0.05% gelatin. Transfection of the expression vectors pSHAG2 (37) and pB42HPV16E7 (27) was performed using the Effectene method (Qiagen, Hilden, Germany). 38 h post-transfection, cells were prepared for indirect immunofluorescence according to standard protocols, including methanol fixation. After incubation with primary antibodies (polyclonal rabbit anti-α-glucosidase antibodies (33) and monoclonal mouse anti-HPV-16 E7 antibody TVG701 (38), respectively), and secondary antibodies (TRITC-conjugated anti-Mouse IgG and FITC-conjugated anti-rabbit IgG from Dianova, Hamburg, Germany), cells were washed and embed-
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**RESULTS**

**Identification of Acid α-Glucosidase as E7-binding Protein**—To identify additional targets for E7, a human cDNA library (31) was screened in a yeast two-hybrid experiment, using the C-terminal domain of HPV-16 E7 as bait (Fig. 1A). In this screen, the cDNA encoding acid α-glucosidase (αGluc, Fig. 1B) was repeatedly isolated. Specific interaction of acid α-glucosidase with wild type E7 but not with two unrelated fusion proteins, LexA-Bicoid and LexA-C-Myc, was confirmed by coexpression in yeast, followed by a selection for leucine prototrophy (Fig. 1C). A lacZ reporter gene system was then applied to map the E7 domains required for the interaction with acid α-glucosidase, using LexA fusion proteins containing isolated subdomains of E7 or specific E7 mutants (Fig. 2A). As shown in Fig. 2B, this experiment revealed that full-length E7 as well as the isolated E7 C terminus strongly interact with acid α-glucosidase, whereas the isolated cd1-(1–17) and cd2-(18–38) subdomains of E7 are inactive in this assay. Similarly, two mutations in the C terminus of E7, GLY58/91 (19) and GLY79/83 (27), strongly reduced the ability of E7 to bind acid α-glucosidase, confirming the conclusion that sequences in the E7 C terminus mediate the binding of acid α-glucosidase. Similar quantities of all fusion proteins were produced in the yeast strains analyzed and cd2) and the putative zinc finger motifs (CXCC) are indicated. E7 mutants used in this report are shown. B, Saccharomyces cerevisiae strain XAY4/gal1-TRP1, containing lacZ::TRP1, was transformed with the plasmid pB42-acid-α-Gluc::TRP1. Plasmids encoding various LexA fusion proteins were coexpressed, as indicated, and β-galactosidase activity was determined. C, LexA fusion proteins from yeast strains used in B were quantitated by Western blotting, using a polyclonal antiserum to LexA (30). D, purified GST or GST-E7 fusion proteins immobilized on glutathione-Sepharose 4B beads were incubated with whole-cell extract from yeast cells, and the amount of B42-HA1-acid-α-Gluc protein that was retained on the beads was determined by direct immunoblotting as described (28), using a polyclonal antiserum to acid α-glucosidase.

![Fig. 2. Mapping of the acid α-glucosidase-binding site in E7.](image)
in Figs. 1C and 2B, and these proteins were stable (Fig. 2C; see also Ref. 30), confirming that the inability of LexA-E7cd1, LexA-E7cd2, and LexA-E7Δ79-83 to promote reporter gene activity is due to the inability of these proteins to interact with acid α-glucosidase.

The yeast fusion proteins were then used to demonstrate the binding of acid α-glucosidase to HPV-16 E7 in vitro. When lysates of yeast cells were incubated with GST HPV-16 E7 fusion proteins, the protein containing acid α-glucosidase fused to the B42 trans-activation domain was specifically retained by GST-E7 but not GST protein alone (Fig. 2D), whereas the isolated B42 domain did not bind to either GST or GST-E7 (data not shown; see Ref. 28). Thus, E7 specifically interacts with the acid α-glucosidase part of the fusion protein.

Direct Physical Interaction between HPV-16 E7 and Acid α-Glucosidase—To determine if E7 can bind to acid α-glucosidase from mammalian cells, purified GST-E7 fusion proteins were incubated with the 110-kDa (precursor) form of acid α-glucosidase, which had been purified to homogeneity from the milk of acid α-glucosidase transgenic mice (39). As is shown in Fig. 3A, purified acid α-glucosidase interacts with high affinity with GST-16E7 protein but not the GST control. This finding indicates that the 110-kDa form of acid α-glucosidase binds directly to the C terminus of E7, and no additional cellular proteins are required. As in the two-hybrid assay (Fig. 2), deletion of amino acids 79-83 in the C terminus of E7 considerably reduced the interaction of E7 with acid α-glucosidase (to 68 ± 5% of the value obtained with wild type E7, see also Fig. 3B), consistent with the C-terminal domain of E7 being involved in the interaction. Replacement of cysteine at position 24 in E7 by glycine nearly abolished the interaction of E7 with acid α-glucosidase (reduction to 14 ± 3% of the value obtained with wild type E7), indicating that sequences in the N terminus of E7 are also essential for a high affinity interaction (Fig. 3A). Together, these results suggest that several domains of the E7 protein are involved in the interaction with acid α-glucosidase. We also analyzed binding of E7 to the mature lysosomal form of acid α-glucosidase, which had been purified to homogeneity from human placenta (41). Upon incubation of the purified 70/76-kDa form of acid α-glucosidase with GST-E7, similar amounts of the enzyme were bound to GST beads and to

![Fig. 3](https://example.com/figure3.png)

**Fig. 3. Differential interaction of E7 with acid α-glucosidase subspecies.** A, the purified 110-kDa form of the acid α-glucosidase protein was incubated with beads containing various GST-E7 fusion proteins as indicated. **Upper panel,** after elution from the beads, bound proteins were separated by gel electrophoresis, and acid α-glucosidase was detected by Western blotting; for comparison, 10% of the acid α-glucosidase input was also loaded on the gel. **Lower panel,** input of the various GST fusion proteins was controlled by Coomassie staining. **B,** GST-E7wt and GST-E7Δ79-83 proteins (40 ng/ml each) were loaded on glutathione-Sepharose 4B beads and incubated with various amounts of purified mature acid α-glucosidase protein, as indicated. The levels of acid α-glucosidase bound to the GST-E7 proteins were analyzed by Western blotting and quantitated by densitometric scanning of the autoradiogram. Percent affinity was plotted against acid α-glucosidase

![Fig. 4](https://example.com/figure4.png)

**Fig. 4. Cytoplasmic localization of HPV-16 E7 in CaSki cells.** CaSki cells were subjected to mild lysis. Subsequently, nuclei were separated from cytoplasm by centrifugation. Nuclear and cytoplasmic fractions were separated by SDS-polyacrylamide gel electrophoresis and probed with antibodies to lamin B, M2 pyruvate kinase, calreticulin, and HPV-16 E7, as indicated. Total cellular lysates were analyzed as controls.
beads containing GST-E7, indicating that this form of the enzyme does not specifically interact with E7 (Fig. 3C).

The 110-kDa form of acid α-glucosidase is found in the endoplasmic reticulum and the Golgi complex (37, 42). Although a fraction of E7 is localized to the nucleus (43), cytoplasmic localization of E7 was also reported (21, 22, 44), suggesting that E7 and acid α-glucosidase may interact in the cytoplasm. To address this point, nuclear and cytoplasmic extracts of E7-expressing cervical carcinoma (CaSki) cells were prepared. As expected, the nuclear protein lamin B was retrieved in the nuclear fraction, whereas both the cytosolic enzyme M2 pyruvate kinase and the ER-specific protein calreticulin were found exclusively in the cytoplasmic fraction, suggesting that a clean separation of nuclear and cytoplasmic proteins had been achieved (Fig. 4). In these experiments, a significant proportion of the E7 protein is retained in the cytoplasm, in keeping with the published literature (21).

To study further the subcellular localization of both proteins and to determine whether E7 and acid α-glucosidase can interact in living cells, the subcellular localization of both proteins was investigated by indirect immunofluorescence analysis. U2-OS cells, a human osteosarcoma cell line with high intrinsic transfection efficiency (35), were transfected with expression vectors for either E7 or glucosidase with the anti-E7 (left panel) or anti-glucosidase antibodies (right panel) is shown. In the right half of the figure, the endogenous acid α-glucosidase protein is revealed by faint green staining. A, staining of cells expressing either E7 or glucosidase is shown. In the right half of the figure, the endogenous acid α-glucosidase protein is revealed by faint green staining. B–D, U2-OS cells were cotransfected with expression vectors for both HPV-16 E7 and acid α-glucosidase. The structures stained with anti-E7 (B) and anti-glucosidase antibodies (C) are colocalized, as shown by costaining (yellow in D).

To analyze further colocalization of E7 and acid α-glucosidase, both proteins were coexpressed by transient transfection. The cells were incubated with antibodies to both proteins and stained with anti-E7 antibodies (Fig. 5B, red fluorescence) and anti-glucosidase antibodies (Fig. 5C, green fluorescence). Both staining patterns were found to overlap, and superimposition of the pictures revealed large areas of colocalization, as revealed by yellow fluorescence (Fig. 5D). Together, these experiments clearly establish that E7 and acid α-glucosidase colocalize in mammalian cells.

E7-dependent Activation of Glucosidase Activity—To determine if E7 can modulate the function of acid α-glucosidase in vivo, we assayed the activity of the enzyme in extracts from 14/2 cells (34), in which expression of the E7 gene can be induced from a dexamethasone-inducible promoter (Fig. 6A). As shown in Fig. 6B, expression of E7 in 14/2 cells resulted in
a roughly 7-fold increase in the substrate affinity of the enzyme in vivo (Table I). In control experiments using normal rat kidney cells, we found that the substrate affinity of acid α-glucosidase was not significantly affected by the addition of dexamethasone to the cells (Fig. 6C), strongly suggesting that the difference in enzymatic activity obtained in 14/2 cells depends on the expression of a functional E7 gene. To assess the ability of the C-terminal deletion mutant E7Δ79–83 to modulate the enzymatic activity of acid α-glucosidase in vivo, a new cell line was constructed in which the HPV-16 E7 mutant E7Δ79–83 is expressed under control of the murine mammary tumor virus promoter. Expression of the E7 mutant in this cell line was verified by Western blotting. We found that both cell lines did not express E7 protein in the absence of dexamethasone, and in cells induced by addition of dexamethasone, the E7 mutant was expressed to a similar level as wild type E7 (Fig. 6A). We found that, in untreated cells, i.e. in the absence of any E7 protein, the activity of acid α-glucosidase was similar if not identical in extracts obtained from 14/2 cells and 14/Δ79–83 cells. In contrast to the results obtained with wild type E7 (Fig. 6B), expression of the E7 mutant did not significantly alter the enzymatic activity of acid α-glucosidase (Fig. 6D and Table I), indicating that the sequence in the C terminus of E7 that mediates binding of E7 to acid α-glucosidase is also required for the modulation of the enzymatic activity.

To analyze if E7 may be able to modulate the activity of acid α-glucosidase in vitro, extracts prepared from non-induced 14/2 cells (E7 not expressed) were incubated with purified recombinant GST-E7 fusion proteins, followed by a determination of the acid α-glucosidase activity. In these experiments, we found

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![Fig. 6. Modulation of acid α-glucosidase activity by E7 in vivo. A, expression level of the E7 protein in the cell lines 14/2 and 14/Δ79–83 after dexamethasone withdrawal (−Dex.) and 4 h after induction of E7 by dexamethasone (5 μg/ml; +Dex.). 200 μg of total cellular lysate was separated on a 12.5% SDS-polyacrylamide gel electrophoresis as indicated, transferred to a nylon membrane, and analyzed with a monoclonal antibody against HPV-16 E7 (Santa Cruz Biotechnology). B, enzymatic activity was measured in 14/2 cells after dexamethasone withdrawal (−Dex.) and 4 h after induction of E7 by dexamethasone (5 μg/ml; +Dex.), respectively. C, activity of acid α-glucosidase was determined in NRK cells either without previous treatment or after incubation with dexamethasone (5 μg/ml) for 4 h. D, enzymatic activity was measured in 14/Δ79–83 cells after dexamethasone withdrawal (−Dex.) and 4 h after induction of E7 by dexamethasone (5 μg/ml; +Dex.), respectively.

| Cell line          | Km (±S.D.) | Significance |
|--------------------|------------|--------------|
| 14/2 wt −Dex       | 4.63 ± 2.86 |              |
| 14/2 wt +Dex       | 0.67 ± 0.20 | p < 0.005    |
| 14/Δ79–83 −Dex     | 6.82 ± 2.20 |              |
| 14/Δ79–83 +Dex     | 7.51 ± 1.53 | NS           |

TABLE I

Modulation of acid α-glucosidase activity in vivo

Extracts of non-induced 14/2 cells (no E7 expressed), dexamethasone-induced 14/2 cells (wild type E7 expressed), non-induced 14/Δ79–83 cells (no E7 expressed), and dexamethasone-induced 14/Δ79–83 cells (mutant E7 expressed) were prepared, and glucosidase activity was measured. Km values were determined, using 4-MUG as substrate in varying concentration. Mean values of at least three independent experiments ± S.D. and the statistical significance are shown. NS, not significant.
that addition of recombinant E7 protein resulted in a significant increase in the substrate affinity of acid α-glucosidase, whereas GST alone had no effect (Table II). Addition of either the Δ79-83 or GLY24 mutant of HPV-16 E7 did not significantly affect the substrate affinity of acid α-glucosidase (Table II). Similarly, the catalytic activity of the purified recombinant 110-kDa form of acid α-glucosidase could be stimulated by purified E7 protein in vitro, whereas E7 mutants Δ79-83 and GLY24 were much less active in this assay. In contrast, the activity of the 70/76-kDa enzyme, which is not bound by E7 (Fig. 3), was not affected by the viral oncoprotein. Taken together, these data suggest that the ability of E7 to bind the 110-kDa form of acid α-glucosidase is required for the modulation of the enzyme activity by E7, and activation of acid α-glucosidase by E7 is independent of any additional cellular proteins.

Modulation of Intracellular Glycogen Levels by E7—As a major function of acid α-glucosidase is the production of glucose from glycogen (45), we wondered whether expression of E7 in our experimental cell line would result in a change of the cellular glycogen content. To analyze this question, intracellular glycogen was determined in 14/2 cells in which E7 expression was either not induced or induced for 4 h. This experiment revealed that expression of E7 for 4 h resulted in the degradation of about 40% of the cellular glycogen content (Fig. 7). To test if increased degradation of glycogen may be related to the observed activation of acid α-glucosidase within the cells (see Fig. 6), conduritol B epoxide, a specific inhibitor of acid α-glucosidase (45), was added to the culture medium of the cells. Although we still noted a significant decrease of the glycogen content under these conditions, glycogen degradation was significantly reduced by conduritol B epoxide treatment. The results of three independent experiments demonstrate that 14.7 ± 3% of the total cellular glycogen is degraded by acid α-glucosidase upon expression of E7 (Fig. 7).

DISCUSSION

We show here that HPV-16 E7 binds directly to the purified 110-kDa form of acid α-glucosidase and increases its normally very low substrate affinity (46) to a level that is even higher than observed for the mature form of the enzyme. For both the physical interaction and the modulation of enzymatic activity, no additional proteins are required, suggesting that E7 can act as a direct allosteric activator of acid α-glucosidase. Expression of wild type E7 but not a C-terminal mutant increases glucosidase activity in vitro, which leads to increased glycogen breakdown in vivo.

Although it is generally assumed that the interaction of HPV-16 E7 with members of the retinoblastoma protein family is critical for its ability to transform mammalian cells, it is now clear that the interaction of E7 with members of the pRb family is not sufficient for cell transformation. Mutations in the C-terminal part of E7, which leave pRb binding intact, render E7 unable to immortalize human keratinocytes (18, 19) and to elicit the formation of warts in an animal model for papillomavirus-associated diseases (47). These findings suggest that domains in the C-terminal part of E7 target additional cellular pathways that are involved in the oncogenic activity of E7 (for recent review see Ref. 48). We have previously identified M2 pyruvate kinase as a new binding partner for HPV-16 E7, and we found that the interaction of E7 with M2-PK changes the catalytic properties of that enzyme (28), suggesting that E7 directly interferes with regulation of the cellular carbohydrate metabolism. Our present results extend these initial findings by showing that E7 directly targets another metabolic enzyme, acid α-glucosidase. Whereas both M2-PK and acid α-glucosidase bind to the C terminus of E7, the sequence requirements for both interactions are different, since E7 mutant GLY24 fails to bind acid α-glucosidase (Fig. 3) but interacts with M2-PK with wild type affinity. Together, these results establish that E7, via its C-terminal domains, controls additional, pRb-independent pathways that probably play a role in cell transformation.

The failure of the mature (70/76 kDa) form of acid α-glucosidase to bind E7 indicates that specific structural properties of the 110-kDa form determine its binding affinity. The 70/76-kDa form of acid α-glucosidase, which cannot bind to E7 in vitro, is found exclusively in the lysosomes, whereas the 110-kDa form is found associated with the nuclear envelope, endoplasmic reticulum, and Golgi complex (42). In biochemical fractionation experiments, a significant proportion of the E7 protein is retained in the cytoplasm (Fig. 4), in keeping with the published literature (21). The immunofluorescence analysis reported here suggests that part of the E7 protein is localized to the endoplasmic reticulum (Fig. 5), where it colocalizes with the 110-kDa form of acid α-glucosidase.

What could be the reason that an oncogenic virus like HPV-16 has evolved a protein, which targets acid α-glucosidase?
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We are grateful to Harald zur Hausen and Peter Bannasch for stimulating discussions, and to Gerald Pfister for technical support. We are grateful to Massimo Tommasino for the TVG701 antibody and for comments on the manuscript.

REFERENCES

1. Weinhouse, S. (1972) Cancer Res. 32, 2007–2016
2. Mazurek, S., Boschek, B., and Eigenbrodt, E. (1997) J. Bioenerg. Biomembr. 29, 315–330
3. Bannasch, P., Klimek, F., and Mayer, D. (1997) J. Bioenerg. Biomembr. 29, 363–373
4. Klimek, F., and Bannasch, P. (1989) Virchows Arch. B Cell Pathol. Incl. Mol. Pathol. 57, 245–250
5. Hacker, H. J., Moore, M. A., Mayer, D., and Bannasch, P. (1982) Carcinogenesis 3, 1265–1272
6. Leloir, L. F. (1967) Natl. Cancer Inst. Monogr. 27, 3–18
7. Bannasch, P., D’Introno, A., Leoneiti, P., Metzger, C., Klimek, F., and Mayer, D. (1998) in Cell Growth and Oncogenesis (Bannasch, P., Kanduc, D., Papa, S., and Tager, J. M., eds) pp. 191–212, Birkhauser Verlag, Basel, Switzerland
8. Galloway, C. K. (1934) Am. J. Surg. 26, 281
9. zur Hausen, H. (1991) Virology 184, 9–13
10. Howley, P. M. (1991) Cancer Res. 51, Suppl. 18, 5019–5022
11. Munger, K., Phelps, W. C., Bubh, V., Howley, P. M., and Schlegel, R. (1989) J. Virol. 63, 4417–4421
12. Phelps, W. C., Yee, C. L., Munger, K., and Howley, P. M. (1988) Cell 53, 539–547
13. Edmonds, C., and Vouwen, K. (1989) J. Virol. 63, 2650–2656
14. Dyson, N., Howley, P. M., Munger, K., and Harlow, E. (1989) Science 243, 934–937
15. Phelps, W. C., Bagchi, S., Barnes, J. A., Raychaudhuri, P., Kraus, V., Munger, K., Howley, P. M., and Nevins, J. R. (1991) J. Virol. 65, 6922–6930
16. Reed, S. I. (1996) Biochim. Biophys. Acta 1287, 151–153
17. Nigg, E. A. (1995) BioEssays 17, 471–480
18. McLayre, M. C., Prattini, M. G., Grossman, S. R., and Laimins, L. A. (1993) J. Virol. 67, 3142–3150
19. Jewers, R., Hildebrandt, P., Ludlow, J., Kell, B., and McCance, D. (1992) J. Virol. 66, 1329–1335
20. Alani, R. M., and Munger, K. (1998) J. Clin. Oncol. 16, 330–337
21. Smotkin, D., and Wettstein, F. O. (1987) J. Virol. 61, 1686–1689
22. Kanda, T., Yama, S., Watanabe, S., Furuno, A., and Yoshike, K. (1991) Virology 182, 723–731
23. Antinore, M. J., Birrer, M. J., Patel, D., Nader, L., and Mecane, D. J. (1996) EMBO J. 15, 1950–1960
24. Zerfass-Thome, K., Zwerschke, W., Mannhardt, B., Tindel, R., Botz, J., and Jansen-Durr, P. (1996) Oncogene 13, 2323–2330
25. Funk, J., Wago, S., Harry, J., Espling, E., Stillman, B., and Galloway, D. (1997) Genes Dev. 11, 2090–2100
26. Berezukataky, E., and Bagchi, S. (1997) J. Biol. Chem. 272, 30315–30340
27. Massimi, P., Pum, D., and Banks, L. (1997) J. Gen. Virol. 78, 2687–2613
28. Zwerschke, W., Munzke, J., Massimi, P., Banks, L., Eigenbrodt, E., and Jansen-Durr, P. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1291–1296
29. Eigenbrodt, E., Reichner, M., Scheeffers, B. U., Scheeffers, H., and Friis, R. (1992) Crit. Rev. Oncog. 3, 91–115
30. Zwerschke, W., Jowig, S., and Jansen-Durr, P. (1996) Oncogene 12, 213–220
31. Gyrus, J., Golemis, E., Chertkov, H., and Brent, R. (1993) Cell 75, 791–803
32. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1999) Current Protocols in Molecular Biology (Janssen, K., ed) John Wiley & Sons, Inc., New York
33. Oude, E. R., Strijland, A., Surya, I., Brouwer, K. E., Kroo, M., Hilkena, J., Hilgers, J., Reuser, A. J., and Tager, J. M. (1984) Eur. J. Biochem. 139, 497–502
34. Croet, T., Morgenstern, C., Crawford, L., and Banks, L. (1989) EMBO J. 8, 513–519
35. Zerfall-Thome, K., Schulze, A., Zwerschke, W., Vogt, B., Helin, K., Bartek, J., Henglein, B., and Jansen-Durr, P. (1997) Mol. Cell. Biol. 17, 407–415
36. Schulze, A., Mannhardt, B., Zerfall-Thome, K., Zwerschke, W., and Jansen-Durr, P. (1996) J. Virol. 70, 2335–2344
37. Hoefkouw, L. H., Hoogeveen, W. M., Kroo, M. A., van, B. J., Reuser, A. J., and Oostra, B. A. (1988) EMBO J. 7, 1697–1704
38. Zatsepena, O., Brasingen, J., Robberson, D., Hajiagabri, N., Blight, K., Ely, S., Hihna, M., Pietikovsky, D., Trendelenburg, M., Crawford, L., and Tommasino, M. (1997) Oncogene 14, 1137–1145
39. Bijvoet, A., Kroo, M. A., Pieper, F. R., Van der Vlet, M., De Boer, H., Van der Ploeg, A., Verbeet, M. P., and Reuser, A. (1998) Hum. Mol. Genet. 7, 1815–1824
40. Fuller, M., Van der Ploeg, A., Reuser, A. J., Anson, D. S., and Hopwood, J. J. (1995) Eur. J. Biochem. 234, 903–909
41. Van der Ploeg, A., Kroo, M. A., Willensen, R., Bruns, N. H., and Reuser, A. J. (1991) J. Clin. Invest. 87, 513–518
42. Wisselaar, H. A., Kroo, M. A., Herrmans, M. M., van, B. J., Reuser, A. J., and Oostra, B. A. (1998) EMBO J. 7, 1697–1704
43. Fujikawa, K., Furuie, M., Uwase, R. I., Maki, H., and Yoshie, O. (1994) Virology 204, 789–793
44. Sato, H., Watanabe, S., Furuno, A., and Yoshike, K. (1989) Virology 170, 311–315
45. Herrmans, M. M., Kroo, M. A., van Beeumen, J., Oostra, B. A., and Reuser, A. J. (1991) J. Biol. Chem. 266, 13507–13512
46. Franci, C., Egna, G., Arribas, R., Reuser, A. J., and Real, F. X. (1996) Biochem. J. 314, 33–40
47. Defeo-Jones, D., Vucocol, G., Haskel, K., Hanák, M., Kiefer, D., McAvoy, E., Ivey-Hoyle, M., Kruma, J., Oliff, A., and Jones, R. (1993) J. Virol. 67, 716–725
48. Zwerschke, W., and Jansen-Durr, P. (2000) Adv. Cancer Res. 78, 1–29
49. Eigenbrodt, E., Gerbracht, U., Masurek, S., Presic, F., and Friis, R. (1994) Biochem. Mol. Aspects of Malignant Cancers 2, 311–385
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J. Biol. Chem. 2000, 275:9534-9541.
doi: 10.1074/jbc.275.13.9534

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