Expression of the human papillomavirus type 16 genome in SK-v cells, a line derived from a vulvar intraepithelial neoplasia

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The SK-v cells, established from a premalignant vulvar lesion, contain human papillomavirus type 16 (HPV-16) sequences integrated at a single cellular site and derive from a cell clone present in vivo. Transcription of the HPV-16 genome in SK-v cells was analysed by cDNA heteroduplex mapping and sequencing, and by RNase mapping. Viral sequences were shown to be transcribed into virus-cell fusion messengers. The two major transcripts have a coding capacity for a truncated E6 protein, an E7 protein and an E1-E4 fusion protein, but differ in their 3' virus-cell junction. Minor transcripts have a coding capacity for a full-length E6 protein and another truncated version of E6. The transcription pattern in the E6-E7 region was found to be the same both in SK-v cells and in CaSki cells, a line derived from an invasive cervical carcinoma. Immunoprecipitation experiments showed that the E6 protein (18K) and, predominantly, the E7 protein (20K) are expressed in SK-v cells as in CaSki cells. The E7 protein was found in a two- to threefold lower amount in SK-v cells, but showing the same half-life (about 1 h).

Introduction

The genomes of certain human papillomaviruses (HPVs), namely HPV-16, -18, -31, -33, -35, -39 and -52, are found associated with a high proportion of genital squamous cell carcinomas and their intraepithelial precursors (Dürst et al., 1983; Boshart et al., 1984; Beaudenon et al., 1986, 1987; Lorincz et al., 1986, 1987; zur Hausen & Schneider, 1987; Yajima et al., 1988), supporting a causal relationship between the infection by specific HPV types and the development of genital tumours. The HPV genomes are generally maintained as episomes in intraepithelial neoplasia and integrated within the host DNA in invasive cancers and in cervical-derived cell lines (Lehn et al., 1985; Schwarz et al., 1985; Dürst et al., 1986; Matsukura et al., 1986; Baker et al., 1987; Beaudenon et al., 1987; Choo et al., 1987; El Awady et al., 1987). Integration usually interrupts the viral genome in the E1-E2 open reading frames (ORFs), while retaining the regulatory region and the E6 and E7 ORFs (Lehn et al., 1985; Schwarz et al., 1985; Dürst et al., 1986; Matsukura et al., 1986; Baker et al., 1987; Beaudenon et al., 1987; Choo et al., 1987; El Awady et al., 1987). Viral transcripts containing sequences from the E6-E7 region are expressed in intraepithelial neoplasia and invasive cancers (Schwarz et al., 1985; Smotkin & Wettstein, 1986; Crum et al., 1988; Shirasawa et al., 1988; Smotkin et al., 1989). Virus-cell fusion transcripts with a coding capacity for the E6 ORF, an E6* ORF created by an internal splicing within E6, and the E7 ORF have been found in cervical carcinoma-derived cell lines and in a case of invasive cancer (Schneider-Gădicke & Schwarz, 1986; Smotkin & Wettstein, 1986; Inagaki et al., 1988). Finally, the E6 and E7 proteins have been detected in cervical-derived cell lines (Androphy et al., 1986; Seedorf et al., 1986; Smotkin & Wettstein, 1986, 1987). These data strongly suggest a role for the integration of HPV sequences and the expression of the E6-E7 region from integrated sequences in the progression of genital tumours. However, the step at which these events occur and the mechanisms by which they contribute to tumour progression are not well understood.

To address these questions we have studied a keratinocyte line, named SK-v, derived from a cell clone present in a premalignant genital lesion (a vulvar intraepithelial neoplasia). SK-v cells contain 10 to 20 HPV-16 genome equivalents, which correspond to a single integrated HPV-16 genome interrupted in the E2-L2 region, amplified along with flanking cellular sequences (Schneider-Maunoury et al., 1987). Viral sequences are integrated on a non-rearranged chromosome 12, in region q14-q15 (Sastre-Garau et al., 1990). In this paper we have analysed the transcription of the integrated HPV-16 genome and the expression of the E6 and E7 proteins in SK-v cells between the 50th and the 70th passages. When injected into nude mice SK-v cells at these subcultures induce slowly growing cysts showing...
the histological features of a carcinoma in situ (unpublished results). We have also compared the mode of expression of the HPV-16 genome in SK-v cells and in CaSki cells, a line derived from an invasive cervical squamous cell carcinoma (Pattillo et al., 1977; Smotkin & Wettstein, 1986).

Methods

Cell culture and RNA extraction. The SK-v keratinocyte line was established in our laboratory from vulvar Bowenoid papules (G. Orth & N. Jibard, unpublished results) and SK-v cells were taken between the 50th and the 70th passages (SK-vl) (Schneider-Maunoury et al., 1987). CaSki cells (Pattillo et al., 1977) were kindly provided by L. Gissmann (Deutsches Krebsforschungszentrum, Heidelberg, F.R.G.) and were used between the 260th and the 280th passages. The cells were grown in Eagle's MEM with Earle's salts (Gibco) supplemented with 0.3 g/l glutamine, 10% foetal calf serum and 10⁻⁶ M-hydrocortisone. Cytoplasmic RNA was isolated from SK-v and CaSki cells, as described (Maniatis et al., 1982). For cDNA synthesis poly(A)⁺ RNA was isolated from SK-v cell cytoplasmic RNA by oligo(dT)-cellulose chromatography, as previously described (Schneider-Maunoury et al., 1987).

cDNA cloning and sequencing. cDNA was synthesized from SK-v cell poly(A)⁺ RNA by the method described by Gubler & Hoffman (1983). The first strand was primed by oligo(dT) and synthesized by avian myeloblastosis virus reverse transcriptase, the RNA strand was then nicked by RNase H and the second cDNA strand synthesized using DNA polymerase I. EcoRI linkers (Boehringer) were ligated to the blunt ends of the cDNA molecules, which were inserted into the EcoRI site of the φgt10 vector (Promega) and packaged in vitro (Huynh et al., 1984). Selection of recombinant phages was performed by plating the phages on Escherichia coli strain C600 (Huynh et al., 1984). Two HPV-16-positive clones were identified by plaque hybridization using 32p-labelled HPV-16 DNA as a probe (Maniatis et al., 1982). For cDNA sequencing the 7-5 kb HindIII fragment of each cDNA clone (which contains the EcoRI cloning site) was subcloned into the pSP64 vector (for clone 1A), or into the pUC vector (for clone 11A) and sequenced (Chen & Seeburg, 1985) using λ gt10 sequencing primers (New England Biolabs). A 1-2 kb EcoRI fragment corresponding to the insert cDNA 1A was subcloned in the M13mp19 vector (Sanger et al., 1980) and internal deletions were obtained with T4 DNA polymerase (Dale et al., 1985), using the cyclone system (International Biotechnologies). Subclones with deletions of different sizes were sequenced (Sanger et al., 1980; Biggin et al., 1983). Comparison was done with the sequence determined by Seedorf et al. (1985), including the modification leading to an uninterrupted E1 ORF (Matsukura et al., 1986; Baker et al., 1987).

Heteroduplex analysis. The plasmid pSK-v, which contains the HPV-16 genome integrated in SK-v cells and the flanking cellular sequences (Schneider-Maunoury et al., 1987), was digested with XbaI or HindII endonucleases and hybridized with HindIII-digested λ gt10 clones 1A and 11A. DNAs were annealed and spread in the presence of 50% formamide at 20 °C (Davis et al., 1971). The heteroduplex molecules were inspected under an electron microscope and measured with a digital length calculator (Numonics Corporation) connected to a Nova 3D computer (Data General Corporation) (Schneider-Maunoury et al., 1987).

RNase mapping. Recombinant plasmids containing subgenomic fragments of the HPV-16 genome in the antisense orientation were obtained by digestion with different restriction enzymes (see below), end-filling by Klenow polymerase and cloning in the Smal site of the pSP65 vector (for probes 57-197, 57-282, 275-477, 500-703 and 312-3498), or between the Psrl and Smal sites of the pSP64 vector (for probe 3498-3692). Recombinant plasmids containing the fragments corresponding to probes 57-197 (a Hpal1—Rsal fragment), 57-282 (a Hpal1—Ndel fragment), 275-477 (a HindI—HindII fragment), 500-703 (a Hpal1—Hpal1 fragment) and 312-3498 (a HindII—Hpal1 fragment) were linearized by BarnHI, and the recombinant plasmid containing the fragment corresponding to probe 3498-3692 (a Hpal1—Psrl fragment) was linearized by EcoRI. The probes were prepared by transcription, as described by Melton et al. (1984), using 32pIUTP (30 TBq/mmol) (Amersham). Transcription products were fractionated on 4% acrylamide-7 M-urea sequencing gels and full-length transcripts were eluted for 90 min at 45 °C (Thierry & Yaniv, 1987). Hybridizations of purified probes (10⁶ c.p.m.) with RNA samples (30 μg) were performed at 45 °C overnight, as described by Melton et al. (1984) and RNase digestions were carried out at 37 °C for 1 h, using RNase A (30 μg/ml) and RNase T1 (3 μg/ml) (Boehringer). Digestion products were treated with Proteinase K (5 μg/ml), phenol-extracted and analysed on 8% acrylamide-7 M-urea sequencing gels (Thierry & Yaniv, 1987).

Immunoprecipitations. Cell labelling and immunoprecipitations were performed as previously described (Smotkin & Wettstein, 1986; Barbosa & Wettstein, 1988). The anti-E6 and anti-E7 rabbit antiserum raised against TrpE-E6 or TrpE-E7 fusion proteins, and the antiserum blocked by the fusion proteins, were kindly provided by D. Smotkin and F. Wettstein (University of California, Los Angeles, Ca., U.S.A.). Briefly, cells were labelled for 1 h in cysteine-free MEM containing 10 μM-methionine, supplemented with 0.5 mCi per 60 mm dish of [35S]cysteine (> 37 TBq/mmol) (Amersham). Cells were lysed, either immediately or, for pulse-chase experiments, after removal of the labelled medium and incubation in normal culture medium for different times (Smotkin & Wettstein, 1986; Barbosa & Wettstein, 1988). Lysates were incubated overnight at 4 °C with 1:100 dilutions of the antiserum (Smotkin & Wettstein, 1986; Barbosa & Wettstein, 1988). Antibody-antigen complexes were collected by binding to Protein-A-Sepharose and analysed by 7-5 to 15% gradient SDS-PAGE. After fixation gels were equilibrated with Autofluor (National Diagnostics Incorporated), dried and autoradiographed (Hyperfilm betamax, Amersham). Densitometric analysis of the autoradiograms was done using an image analysis technique (G. Masson, unpublished results).

Results

Construction of the cDNA clones

Our previous studies had shown that two major transcripts (1 kb and 1.2 kb), containing E6 and E7 sequences, are expressed from the HPV-16 genome in SK-v cells (Schneider-Maunoury et al., 1987). To characterize these transcripts further a cDNA library was constructed from poly(A)⁺ RNA of SK-v cells, using the λ gt10 vector (Huynh et al., 1984) and screened with a 32p-labelled HPV-16 DNA probe. Two clones out of 400000 were positive (clones 1A and 11A), which is consistent with the low amount of HPV-16 transcripts usually detected in SK-v cells (Schneider-Maunoury et al., 1987).

Heteroduplex analysis of the cDNA clones

After digestion of the cDNA clones with HindIII a 7.5 kb fragment was shown to contain a 1.2 kb EcoRI insert.
corresponding to the cDNA (data not shown). Heteroduplex molecules were formed between the HindIII-digested cDNA clones and the plasmid pSK-v linearized by XbaI (Fig. 1a), which contains the HPV-16 genome integrated in the SK-v cells and flanking cellular sequences (Schneider-Maunoury et al., 1987). Heteroduplex molecules showing four double-stranded regions separated by three loops were observed with both cDNA clones, as illustrated for clone 1A (Fig. 1b and c). To map the duplex segments heteroduplex molecules were formed between the HindIII-digested cDNA clones and fragments obtained by treatment of pSK-v with HindII (Fig. 1a), including a 7-4 kb fragment that contains the E6–E7 region and a 3-7 kb fragment that contains the E4 ORF and the 3' virus–cell junction (data not shown) (Schneider-Maunoury et al., 1987). Measurements of the heteroduplex molecules indicated that the first exon is located in the E6 ORF, the second exon spans the 3' part of the E6 ORF and the entire E7 ORF, the third exon spans the E4 ORF (Fig. 1a, c), whereas the E1 ORF and the 5' end of the E2 ORF are spliced out. The fourth duplex region is located within 3' flanking cellular sequences (Fig. 1a, c) and is separated from the third exon by a short region of mismatch, instead of a loop (Fig. 1b, c), indicating that the cellular splice acceptor is not located within this region of pSK-v, but is downstream.

**Nucleotide sequence of the cDNAs**

We established the complete nucleotide sequence of one cDNA clone (clone 1A) and parts of the second (clone 11A). The sequence of the coding strand (equivalent to the mRNA sequence) of clone 1A is shown in Fig. 2. The cDNA is 1154 nucleotides long. Only one nucleotide change (C to T at nucleotide 3410 of the HPV-16 genome, i.e. the third nucleotide of a threonine codon) was observed between the viral part of the cDNA and the prototype HPV-16 sequence (Seedorf et al., 1985). This change is conservative for the amino acid sequence of the E4 ORF.

The first nucleotide of both cDNAs corresponds to position 173 of the HPV-16 sequence. The cDNAs probably lack the information for the 5'-terminal part of the corresponding mRNA, since no initiation could be detected at this site by RNase mapping experiments (see below). The first intron (splice donor at nucleotide 226, splice acceptor at nucleotide 409) is located within E6, yielding a truncated E6* ORF; the third codon after the splice acceptor is a stop codon (Fig. 2). The second splice (donor at nucleotide 880, acceptor at nucleotide 3358) joins in frame the eighth codon of E1 to the ninth codon of E4, thus creating an E1–E4 ORF. The third splice (donor at nucleotide 3632) joins viral sequences downstream of the end of E4 to cellular sequences (Fig. 2). Thus, the corresponding mRNA contains at least three ORFs: E6*, E7 and an E1–E4 fusion ORF. No other ORF with an initiation codon at its 5' end was found.

The 3' end of the cDNA is composed of cellular sequences. No poly(A) tail was observed in the cDNAs, but a polyadenylation signal (AATAAA) was found at their 3' end (Fig. 2), suggesting that only a limited part of the mRNA had been removed during cDNA synthesis. The sequence of the cellular part of the cDNA was compared to the EMBL nucleotide sequence Data Library (Lipman & Pearson, 1985). From nucleotide 802 to nucleotide 1055, the cDNA contains 253 nucleotides of an Alu repeat (Jurka & Smith, 1988; Schmid & Jelinek, 1982). It is likely that this repeat accounts for the hybridization observed in heteroduplex experiments between the cellular part of the cDNA and pSK-v, which
contains repetitive human sequences (Schneider-Maunoury et al., 1987).

No difference was found between the cDNAs in the regions for which both nucleotide sequences were established (positions 1 to 459, 645 to 866, 920 to 1154).

### Mapping of the 5' exons of the HPV-16 transcripts

To map the 5' ends of the HPV-16 transcripts and to characterize mRNA species present in low amounts, we performed RNase mapping of SK-v cell cytoplasmic RNAs (Melton et al., 1984; Thierry & Yaniv, 1987). Four probes spanning the E6–E7 region (Fig. 3b) were used. The CaSki cell line, for which the HPV-16 transcripts have been mapped (Smotkin & Wettstein, 1986), was used as a control.

A probe spanning the 5' end of E6 (probe 57-197 in Fig. 3b) revealed a set of close bands for both SK-v cells (Fig. 3a, lanes 1 and 2) and CaSki cells (data not shown). This signal could correspond to heterogeneous initiations at nucleotides 97 to 109 of the HPV-16 genome, i.e. on either side of the second ATG of E6 (nucleotide 104). Such heterogeneous initiation has also been reported for the HPV-18 transcripts in carcinoma cell lines (Schneider-Gädicke & Schwarz, 1986).

To map the exon–intron boundaries in the E6–E7 region three probes were used (probes 57-282, 275-477 and 500-703 in Fig. 3b). The same three mRNA species were mapped in both cell lines (Fig. 3a, lanes 3 to 12 and 3c). The major species contains a truncated E6* ORF, obtained by an internal splicing within the E6 ORF. The splice donor was localized at nucleotide 226 after hybridization with probe 57-282 (a band of 128 nucleotides) (Fig. 3a, lanes 4 to 6) and the splice acceptor was localized at nucleotide 409 after hybridization with probe 275-477 (a band of 69 nucleotides) (Fig. 3a, lanes 8 and 9). This species also contains the whole E7 sequences covered by the 500-703 probe (a band of 203 nucleotides) (Fig. 3a, lanes 11 and 12), which corresponds to the SK-v cDNA sequence and to the major HPV-16 transcript previously characterized in CaSki cells (Smotkin & Wettstein, 1986). A minor unspliced species contains the entire E6 ORF (bands of 180 and 200 nucleotides with probes 57-282 and 275-477, respectively; Fig. 3a, lanes 4 to 6 and 9) and the E7 sequences (a band of 203 nucleotides with probe 500-703) (Fig. 3a, lanes 11 and 12). In CaSki cells the band of 200 nucleotides is detectable only at long exposure times (data not shown). A third species is represented by a band of 169 nucleotides, observed both in SK-v and CaSki cells, with
Fig. 3. RNase mapping of the 5' exons of the HPV-16 transcripts in SK-v and CaSki cells. (a) RNA preparations from SK-v cells (lanes 2, 4, 5, 9 and 11), or from CaSki cells (lanes 6, 8 and 12) were hybridized with 32p-labelled HPV-16 subgenomic 57-197 (lane 2), 57-282 (lanes 4, 5 and 6), 275-477 (lanes 8 and 9) and 500-703 (lanes 11 and 12) RNA probes and the hybrids were digested with a mixture of RNases A and T1. Lanes 1, 3, 7 and 10 show undigested probes (10³ c.p.m./slot), lane 5 shows a partial digestion of probe 57-282. (b) Location of the probes in the HPV-16 genome. The first and the last nucleotides of each probe are given. The HPV-16 ORF E6, the 5' end of E7 and the corresponding ATG translation initiation codons are represented. (c) Map of the 5' ends of the three mRNA species detected in SK-v and CaSki cells. The nucleotide positions of the initiation sites and of the splice donors and acceptors are given. Dashed lines represent spliced-out sequences.

HPV-16 genome expression in SK-v cells

Mapping of the exons at the virus-cell junction

At the virus-cell junction two major mRNA species were identified in SK-v cells, using two probes (probes 3212-3498 and 3498-3692 in Fig. 4b). Both species have a splice acceptor at nucleotide 3358, but differ in their 3' end. The first transcript spans the virus-cell junction (nucleotide 3662) and the second one contains a splice donor located in viral sequences, downstream of the end of E4 (nucleotide 3632). Only the latter virus-cell junction has been detected by cDNA sequencing.

Expression of the E6 and E7 proteins

To compare the expression of the E6 and E7 proteins in SK-v and in CaSki cells we performed immunoprecipitations from labelled cellular extracts, using antisera to TrpE-E6 (Fig. 5a) and TrpE-E7 (Fig. 5b) fusion proteins (Smotkin & Wettstein, 1986). In both cell lines the anti-E6 antiserum precipitated an 18K protein (Fig. 5a), a size consistent with that of the E6 protein previously identified in CaSki and SiHa cells (Androphy et al., 1986). The relative amounts of the E6 protein in SK-v and CaSki cells varied in two independent experiments (0.2 and 2.5), as evaluated by densitometric analysis of the autoradiograms. A 20K protein was precipitated in both cell lines with the anti-E7 antiserum (Fig. 5b), which is the size expected from previous studies of CaSki and SiHa cells (Smotkin & Wettstein, 1986, 1987). In two independent experiments the amount of E7 protein was two- to threefold higher in CaSki cells than in SK-v cells (Fig. 5b). The high signal observed for E7 protein, as
Fig. 4. RNase mapping of the virus-cell junctions of the HPV-16 transcripts in SK-v cells. (a) SK-v cell RNA preparations were hybridized with 32P-labelled HPV-16 subgenomic 3212-3498 (lane 2) and 3498-3692 (lane 4) RNA probes. Lanes 1 and 3 show undigested probes (10^3 c.p.m./slot). (b) Location of the probes at the virus-cell junction. The HPV-16 ORFs E2 and E4 are represented by open boxes and the 3' part of E2 deleted in SK-v cells (Schneider-Maunoury et al., 1987) is represented by a dashed box. The virus-cell junction (nucleotide 3662) (arrow) and the extremities of the probes are indicated. (c) Map of the exons at the virus-cell junction. The nucleotide positions of the splice donors and acceptors are given and dashed lines represent spliced-out sequences.

Fig. 5 Expression of the E6 and E7 proteins. (a) Extracts from CaSki cells (lanes 1 and 2) and SK-v cells (lanes 3 and 4) were immunoprecipitated with an anti-E6 antiserum (lanes 1 and 3), or with the anti-E6 antiserum blocked by the fusion protein (lanes 2 and 4) (Smotkin & Wettstein, 1986). The arrow points to the position of the 18K E6 protein. (b) Extracts from CaSki cells (lanes 1 and 2) and SK-v cells (lanes 3 and 4) were immunoprecipitated with an anti-E7 antiserum (lanes 1 and 3), or with the anti-E7 antiserum blocked by the fusion protein (lanes 2 and 4). The arrow points to the position of the 20K E7 protein. (c) Extracts from labelled SK-v cells harvested immediately (lane 1), or after a 1 h (lane 2), 2 h (lane 3) or 4 h (lane 4) chase, were immunoprecipitated with the anti-E7 antiserum. The arrowhead points to the position of the E7 protein. (d) Extracts from labelled CaSki cells harvested immediately (lane 1), or after a 1 h (lane 2), 2 h (lane 3) or 4 h (lane 4) chase, were immunoprecipitated with the anti-E7 antiserum. The arrowhead points to the position of the E7 protein. The half-life of the E7 protein was determined from densitometric scanning of autoradiograms.

compared to E6 protein, allowed the study of the metabolic stability of the E7 protein by pulse-chase experiments (Fig. 5c, d). The half-life of the E7 protein was 54 min and 63 min for SK-v and CaSki cells, respectively, within the range of the values previously reported for CaSki and SiHa cells (Smotkin & Wettstein, 1987).
Discussion

Our previous studies had shown that the SK-v cells, established from a premalignant lesion, derive from a cell clone present in vivo, which contained HPV-16 sequences integrated into the cellular DNA (Schneider-Maunoury et al., 1987). The integration pattern, characterized by the disruption of the E2 gene and the conservation of the regulatory region and of the E6 and E7 ORFs, is similar to that observed in cervical cancers and derived cell lines (Lehn et al., 1985; Schwarz et al., 1985; Dürst et al., 1986; Matsukura et al., 1986; Baker et al., 1987; Beaudenon et al., 1987; Choo et al., 1987; El Awady et al., 1987). These data indicated that integration of HPV-16 sequences could be an early event of tumour progression (Schneider-Maunoury et al., 1987).

In this paper we have analysed the transcription of the HPV-16 genome and the expression of the E6 and E7 proteins in SK-v cells, as compared with that observed in CaSki cells, a line derived from an invasive carcinoma. The E6-E7 region is likely to be important for the oncogenic properties of HPV-16. This region is transcribed in invasive cervical cancers and in derived cell lines (Schwarz et al., 1985; Schneider-Gädicke & Schwarz, 1986; Smotkin & Wettstein, 1986; Inagaki et al., 1985; Shirasawa et al., 1988; Smotkin et al., 1989) and is required for immortalization of primary keratinocytes in culture (Dürst et al., 1987; McCance et al., 1988; Woodworth et al., 1988). The HPV-16 E7 gene cooperates with the ras oncogene for the transformation of primary epithelial cells (Crook et al., 1988; Phelps et al., 1988). The E7 protein has homologies with the adenovirus E1A oncoprotein (Phelps et al., 1988) and, like E1A protein, is able to form complexes with the product of the retinoblastoma gene, a tumour suppressor gene (Dyson et al., 1989).

Three transcripts have been characterized in the E6-E7 region in SK-v cells and are initiated between nucleotide positions 97 and 109 of the HPV-16 genome, i.e. on either side of the second ATG of E6. The major transcript contains the E6* ORF, created by an internal splicing within E6, the E7 ORF and an E1–E4 fusion ORF. The two minor species contain a complete E6 ORF and another truncated version of E6, respectively. The same transcription pattern was found in CaSki cells and corresponds to that previously characterized by Smotkin & Wettstein (1986). Interestingly, the nucleotide sequence of the cDNA in the E6–E7 region disclosed no difference with the published sequence of the HPV-16 genome cloned from a cervical cancer (Seedorf et al., 1985). This may indicate that tumour progression does not involve point mutations in the transforming genes.

At the virus–cell junction two major species were identified by RNase mapping in SK-v cells. Thus the two major mRNA species containing E6 and E7 sequences (1 kb and 1-2 kb) found by Northern blot hybridization (Schneider-Maunoury et al., 1987) most probably correspond to heterogeneity in the 3′ cellular sequences. One of these virus–cell junctions was characterized by cDNA sequencing and was shown to contain cellular repetitive sequences, as also observed for HPV-18 transcripts in carcinoma-derived cell lines (Schneider-Gädicke & Schwarz, 1986; Inagaki et al., 1985). Recent studies have shown that cellular sequences can enhance the transforming potential of the HPV-16 E6–E7 region (Le & Defendi, 1988) and it has been postulated that cellular sequences stabilize the viral messenger RNAs (Schneider-Gädicke & Schwarz, 1986; Le & Defendi, 1988).

The E6 and E7 proteins have been detected in both SK-v and CaSki cells. The E7 protein is most likely translated from messenger RNAs initiated after the second AUG of E6. After [35S]cysteine labelling of the cells the E7 protein was detected in larger amounts than the E6 protein, which may reflect the low abundance of the transcripts encoding the E6 protein in both cell lines. The level of the E7 protein was found to be two- to threefold higher in CaSki cells than in SK-v cells and we showed that this is not due to a different metabolic stability. When injected into nude mice at the passages studied SK-v cells induce slowly growing cysts with the features of a Bowen’s carcinoma in situ, whereas CaSki cells induce slow-growing necrotizing invasive squamous cell carcinomas (unpublished data). Whether the E7 level is related to differences in the tumorigenicity of SK-v and CaSki cells will remain a matter of speculation, as long as the same differences have not been demonstrated in tumours induced in nude mice.

In conclusion, the study of SK-v cells suggests that the mode of expression of the viral genome considered as characteristic of the malignant cells, i.e. the expression of the E6 and E7 genes from virus–cell fusion transcripts, can be observed at intraepithelial stages of the progression of genital tumours. This stresses the requirement for additional events to allow progression of intraepithelial neoplasia towards invasive carcinoma.

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References


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