PROPERTIES OF SYRIAN HAMSTER CELLS TRANSFORMED BY HUMAN PAPILLOMAVIRUS TYPE 16

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Received December 3, 1996

Summary. – Adult Syrian hamster kidney cells were transfected with a mixture of plasmids containing human papillomavirus type 16 (HPV16) E6/E7 open reading frames (ORFs), activated Ha-ras gene and neomycin resistence gene. From these cultures two lines were isolated which were oncogenic for newborn and 5-day-old but not for 3-week-old hamsters. Sublines oncogenic for 3-7-week-old hamsters were derived from tumours formed in animals inoculated within 5 days of birth. The cells contained HPV16 DNA in an integrated form and HPV16 transcripts. The transcript patterns in low and high oncogenicity sublines were different. Very few tumour-bearing animals possessed antibodies reactive with E6- and E7-derived synthetic peptides. On the other hand, a majority of these animals gave a positive reaction in the lymphoproliferation assay with either E6 and E7 peptides or extracts from the transformed cells.

Key words: human papillomavirus; hamster cells; transformation; immune reactions

Introduction

HPV16 is strongly associated with cervical cancer (Dürst *et al.*, 1983). The E6 and E7 genes of this virus are expressed in HPV16 DNA-positive cervical cancers and cell lines derived from them (Schwartz *et al.*, 1985). The products of these two genes are apparently responsible for the cell transformation and the maintenance of the transformed state (for recent reviews see Mc Dougall, 1994; Tomassino and Crawford, 1995). Recently, in rats and mice, the immunity against challenge with HPV16-transformed syngeneic cells has been induced by recombinant vaccinia viruses (VVs) expressing E6 and E7 genes or by administration of HPV16-transformed but non-oncogenic cells (Chen *et al.*, 1991,1992; Meneguzzi *et al.*, 1991). These results have raised hopes that it might be possible to develop recombinant VV vaccines or other means for prophylaxis and/or treatment of cervical cancer. Still, a number of questions of basic importance for understanding the immune reactions against HPV-induced tumours remain to be clarified. To be able to study various aspects of anti-HPV tumour immunity, we have recently developed model systems in Syrian hamsters and mice. In this first paper of a series we report on the isolation and some properties of HPV16-transformed hamster cells. To the best of our knowledge, this is the first report on transformation of hamster cells by HPV.

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Abbreviations: aa = amino acid; BSA = bovine serum albumin; DMEM = Dulbecco's modification of Eagle's Medium; DTT = dithiothreitol; HPV16 = human papillomavirus type 16; nt = nucleotide; ORF = open reading frame; PCR = polymerase chain reaction; PBS = phosphate-buffered saline; RT = reverse transcriptase; SI = stimulating index; VV = vaccinia virus

Materials and Methods

Animals and cell cultures. Syrian hamsters (Charles Rivers, Germany) were used for preparing tissue cultures and for oncogenicity tests. Secondary cultures from adult hamster kidney cells were used for transformation experiments. In some experiments

HEF cells, a continuous, spontaneously transformed cell line derived from hamster embryo fibroblasts (Kutinová *et al.*, 1975), and HaCaT cells (Boukamp *et al.*, 1988), were used as negative controls. CaSki cells derived from an HPV16 DNA-positive cervical cancer (Baker, 1983) served as a positive control. For both primary and secondary cultures, Dulbecco's modification of Eagle's Medium (DMEM) (Sevac, Prague) supplemented with 10% of foetal calf serum was used. The established cell lines were grown in EPL medium (Sevac, Prague) (Kutinová and Vonka, 1978).

Plasmids. p16HHMo carrying HPV16 E6/E7 ORFs and a portion of E1 ORF (Vousden *et al.*, 1988), pEJ6.6 containing activated Ha-ras gene (Parada *et al.*, 1982), and pAG60 carrying the neomycin resistence gene (Colbere-Garapin *et al.*, 1981) were used. They had been kindly donated by Drs. K. Vousden (Ludvig Institute for Cancer Research, London), M.Dürst and F.Rössl (both DKFZ, Heidelberg), respectively. pEA16E6 containing HPV16 E6 ORF (nt 24-654 in *Eco*RI and *Bam*HI sites of pEAgpt plasmid) was obtained through the courtesy of Dr. M. Dürst.

Transfection experiments. Subconfluent secondary hamster kidney cell cultures grown in 60 mm plastic Petri dishes (Nunc, Denmark) were co-transfected with 10 μg of plasmid DNA composed of a mixture of p16HHMo, pEJ6.6 and pAG60 plasmids (1:1:1, w:w:w) using lipofectin kit (Transfection Reagent DOTAP, Boehringer, Mannheim) according to the manufacturer's instructions. Parallel cultures were transfected with combinations of two plasmids and some were mock-transfected. Both before and after transfection the cells were cultivated in DMEM supplemented with 10% of foetal calf serum (Sigma). After reaching confluency the cultures were split 1:2 and 200 μg/ml G418 (Sigma) was added.

DNA extraction. High molecular mass DNA was extracted using the sodium dodecyl sulphate (SDS)-proteinase K-phenol-chloroform method of Blin and Stafford (1976).

RNA extraction. Total RNA was extracted using the acid guanidium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987).

Southern and Northern blot analyses. The Southern blot analysis was performed following the original protocol (Southern, 1975) but using a vacuum transfer equipment (Vacu Gene TMxL, Pharmacia, Sweden). The Northern blot analysis was carried out as follows. Twenty μg of total RNA in loading buffer containing 50% (v/v) formamide (Sigma), 17.8% (v/v) formaldehyde (37% solution, Sigma) in 10 mmol/l MOPS buffer (Sigma), 5 mmol/l sodium acetate, 1 mmol/l EDTA, and 1% (v/v) Ficoll 400 (Pharmacia) was denatured for 10 mins at 65°C. The denatured RNA was electrophoresed on 1% agarose gel with marker RNAs (Sigma) and transferred to Hybond-N nylon membrane (Amersham) in 20xSSC buffer (20xSSC: 3 mol/l NaCl, 0.3 mol/l sodium citrate). The membrane was baked at 80°C for 2 hrs, then used for hybridization.

Southern hybridization of DNA was performed in 5xSSPE (1x SSPE: 0.18 mol/l NaCl, 10 mmol/l NaH_2PO_4, 1 mmol/l EDTA, pH 7.7), 5xDenhardt's solution (1xDenhardt's solution: 2% (w/v) BSA, 2% (w/v) Ficoll, 2% (w/v) polyvinylpyrrolidone), 0.5% SDS and 20 μg/ml calf thymus DNA denatured by boiling for 10 mins. The membrane was prehybridized for 1 hr. The DNA probe was prepared as follows. Plasmid pEA16E6 was digested with *Eco*RI + *Bam*HI (Amersham). The resulting 630 bp fragment (HPV16 nt 24-654) was purified from agarose using a DNA purification matrix kit (Bio-Rad) and labelled with [alpha^{32}P]dCTP (Amersham) to the specific activity of 1x10^8 dpm/μg using random priming method (Feinberg and Vogelstein, 1983). The denatured ^{32}P-labelled probe was added to a final concentration of 1x10^6 dpm/ml and the incubation was continued overnight. The membrane was washed twice in 2xSSC with 0.1% SDS at room temperature for 10 mins, and then twice in 1xSSC with 0.1% SDS at 65°C for 30 mins. The membranes were exposed to X-ray film (Fuji,Rx). Northern hybridization was carried out at 42°C under the above conditions with additional 50% formamide. For hybridization with RT-PCR products the oligoprobe (see above) was prepared as follows: 50 pmoles of the oligonucleotide was end-labelled with [gamma-^{32}P]dATP (Amersham) using T4 polynucleotide kinase (Amersham) in 50 mmol/l Tris-HCl pH 7.6, 10 mmol/l $MgCl_2$, 5 mmol/l DTT and 0.1 mmol/l EDTA, at 37°C for 30 mins, to the specific activity of 1x10^7 dpm/pmol. Prehybridization was performed at 60°C for 1 hr in 5xSSC, 1% SDS, 20 mmol/l sodium phosphate pH 7.5 and 200 μg/ml calf thymus DNA denatured by boiling for 10 mins. Hybridization with denatured labelled probe (1x10^6 dpm/ml) was carried overnight under the same conditions as the prehybridization. Washing was performed twice in 2xSSC with 0.1% SDS at 60°C. The membranes were exposed to X-ray film (Fuji, RX) overnight.

Polymerase chain reaction (PCR). Ten ng of DNA was subjected to PCR using HPV16 E6-specific E6R and E6C primers (Johnson *et al.*, 1990). PCR was performed under the same condition as reverse transcriptase-PCR (RT-PCR, see below).

RT-PCR. One μg of total RNA in 20 mmol/l Tris-HCl pH 8.3, 50 mmol/l KCl, 2.5 mmol/l $MgCl_2$ and 0.1 mg/ml bovine serum albumin (BSA) was treated with 1 U of RNAase-free DNAase I (Sigma) in total volume of 10 μl for 25 mins at room temperature and then 1 μl of 20 mmol/l EDTA was added to the reaction mixture. After 10 mins of incubation at 65°C, the first strand of cDNA was synthesized according to the protocol of Frohman *et al.* (1988), using 18 U of reverse transcriptase (RAV2RT, Amersham) and 25 pmoles of oligo(dT)-primer, 5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3', in a total volume of 20 μl. The reaction was performed at 41°C for 2 hrs. Then 2.5 μl of the reaction mixture was used for HPV E6-specific PCR. DNAase- treated RNA untreated with reverse transcriptase was used in parallel to evaluate the possible contamination of the RNA preparations by DNA. PCR amplification was performed in a total volume of 100 μl containing 10 μl of 10xPCR buffer (USB), 200 μmol/l each of dATP, dGTP, dCTP and dTTP, 25 pmoles of forward and backward primers (E6 R: 5'-GCAAGCAACAGTTACT GCGA-3'(nt 201-220) and C: 5'-GTTGTCTCTGGTTGCAAATC-3' (nt 620-601), (Johnson *et al.*, 1990) and 2.5 U of Taq polymerase (USB). After an initial cycle of denaturation at 94°C for 5 mins, annealing at 55°C for 1 min and extension at 72°C for 2 mins, a total of 38 cycles of amplification were performed. Each cycle consisted of denaturation (94°C, 1 min) primer annealing (55°C, 1 min) and extension (72°C, 2 mins). Final annealing (55°C, 1 min) and elongation steps (72°C, 7 mins) were performed after the last cycle. Amplified products were directly analyzed on 3% agarose gels using PCR marker (USB) and

then transferred to nylon membrane (Hybond-N, Amersham). For the hybridization (see below), a labelled internal oligonucleotide probe, Vera 4,5'-TCCATGCATGATTACAGCTG-3' (HPV16, nt 570-551), kindly donated by von Knebel-Doeberitz (DKFZ, Heidelberg), was employed. A mixture containing all reagents except the RNA template and mixtures containing all reagents including the RNA template but lacking reverse transcriptase served as controls.

Oncogenicity tests. Freshly trypsinized cells were washed three times with PBS and injected subcutaneously in the dorsal area of either newborn (within 24 hrs after delivery), 5 day-old or 3-7-week-old Syrian hamsters.

Antibody tests. The presence of antibodies in hamster sera was tested in peptide-based enzyme-linked immunosorbent assay (ELISA). Three peptides corresponding to the N-terminal portion of HPV16 E7 (20 amino acids (aa) long with 10 aa overlap) denoted E7/1 (aa 1-20), E7/2 (aa 11-30) and E7/3 (aa 21-40), and three peptides derived from HPV16 E6 (25 aa long) denoted E6/2 (aa 16-40), E6/8 (aa 106-130) and E6/10 (aa 134-158) were used as peptide antigens. Assays with hamster sera diluted 1:25 were performed as described previously (Hamšíková *et al.*, 1994) with the following modification. After an incubation with hamster sera, rabbit anti-hamster IgG (Sigma) diluted 1:2000 was added for 1 hr at 37°C, and the plates were washed and incubated with horseradish peroxidase-conjugated swine anti-rabbit IgG (SEVAC, Czech Republic) diluted 1:2000 for 1 hr at 37°C. The colour reaction (A_{492}) was determined in a Titertek Multiscan MCC 340 spectrophotometer (Flow Laboratories). Control sera were tested on each plate.

Lymphoproliferative assay. Single-cell suspensions were prepared from spleens of hamsters with K3/II induced tumours. Mononuclear cells were separated by the method of Böyum (1968) on a Ficoll-Isopaque gradient. Adherent cells were depleted by plastic adherence (incubation for 16 hrs at 37°C). The remaining cells (1-2 x 10^5 in 200 µl) were cultivated in RPMI 1640 medium supplemented with 10% heat-inactivated foetal calf serum, 15 mmol/l HEPES buffer, 2 mmol/l glutamine and 40 mmol/l gentamycin in 96-well round-bottom plates (Nunc, Denmark) either in the presence of mixtures of HPV16 E6- and E7-derived peptides or in the presence of extracts from HPV16-transformed H9 cells and the control HPV16 DNA-free HEF cells. The E7 peptide mixture consisted of peptides E7/2 (aa 11-30), E7/3 (aa 21-40), E7/5 (aa 41-60), E7/6 (aa 51-70) and E7/8 (aa 81-98). The E6 peptide mixture consisted of E6/1 (aa 1-25), E6/4 (aa 46-70), E6/5 (aa 61-85), E6/6 (aa 76-100), E6/7 (aa 91-115), E6/8 (aa 106-130), E6/9 (aa 121-145) and E6/10 (aa 134-158). Each well received peptide mixture containing 5 µg of each peptide. Cell extracts were prepared as for the Western blot analysis. Fifty µl of these extracts diluted 1:10 was added per well. After 6 days of cultivation at 37°C the cultures were pulsed with 0.04 MBq of [^{3}H]-thymidine per well for 18 hrs at 37°C and harvested on glass fiber filters. Each antigen was tested in triplicate. Incorporation of radioactivity was measured in a liquid scintillation counter. The proliferation activity was expressed in terms of stimulating index (SI) (SI = cpm in stimulated culture/cpm in control culture). An SI equal to or exceeding 2.0 was considered a positive result. In proliferation assays with cell extract antigens, the cpm values in the presence of HEF-derived antigen exceeded, in several instances, those detected in the cultures kept without any antigen; i.e. the SI exceeded 1.0. Therefore when evaluating results with the H9-derived antigen also the values obtained with HEF antigen were taken into consideration. Only those animals whose SI in the presence of H9 antigen exceeded those obtained in the presence of HEF extract by a value of at least 1.0 were considered positively reacting.

Results

Isolation of transformed cells

The transfection was performed as described in Materials and Methods. After 18 days of cultivation in the presence of G418, 3-13 colonies per dish were observed in cultures transfected with all three plasmids. On subpassage, these cells grew well and after a period of crisis two lines, denoted 5A and H9, of rapidly growing cells were established. In cultures transfected with pAg60 in combination with either p16HHMo or pEJ6.6, 0-1 colony per dish was observed. The growth of these colonies was only transient and no continuous cell lines were obtained.

5A cells from the 8th passage were inoculated subcutaneously into newborn and 5-day-old hamsters (10^6 cells in 0.1 ml) or 3- week-old animals (5 x 10^6 in 0.5 ml). Tumours appeared in animals inoculated within 24 hrs of delivery (in 4 of 10) and on day 5 (in 4 of 12) but not in the older animals. From one tumour a cell line was isolated which was denoted K3/I. These cells were found oncogenic for 3-week-old and older animals. From a rapidly growing tumour induced by K3/I cells another cell line was isolated, which was denoted K3/II. When injected subcutaneously to 3-5-week-old or even older animals, these cells induced rapidly growing tumours 2-4 weeks after inoculation, depending on the cell dose administered. H9 cells exhibited similar properties. They were oncogenic for newborn hamsters (7 of 11) but not for 3-week-old animals. Cell lines denoted H9/I and H9/II were established in the same way as the K3/I and K3/II cells.

Most of the subsequent oncogenicity tests were done with K3/II cells. A large stock of these cells was prepared and kept frozen in liquid nitrogen. All subsequent experiments were done with the third passage of rethawed cells. The results of six repeated experiments in which different doses of K3/II cells were administered to 3-7-week-old hamsters are summarized in Table 1. In these experiments 1 TID_{50} ranged from less than 10 to $10^{2.5}$ cells. It is noteworthy that except the first and the third experiment the oncogenic effect was more marked with the lower than the higher doses of cells in all experiments. This zone phenomenon makes calculation of 1 TID_{50} inappropriate. It was the source of considerable difficulties in protection tests (to be published).

Table 1. Tumorigenicity of K3/II cells in hamsters

No. of cells administered	Exp. No.					
	1[a]	2[b]	3[c]	4[c]	5[a]	6[c]
10^6	3/3	NT	NT	NT	NT	NT
10^5	2/2[d]	NT	3/3	NT	3/3	2/3
10^4	3/3	0/3	3/3	1/3	2/3	2/3
10^3	3/3	3/3	3/3	2/3	2/3	3/3
10^2	3/3	3/3	1/3	2/3	3/3	3/3
10^1	3/3	1/3	0/3	1/3	0/3	2/3
1 TID_{50} (log)	–<0.5	2.2	2.2	2.5	2.2	1.5

[a]Carried out in 3-week-old hamsters.
[b]Carried out in 5-week-old hamsters.
[c]Carried out in 7-week-old hamsters.
[d]One hamster died on day 5 post inoculation without tumour.
NT = not tested.

Presence pf HPV16 DNA in cell lines

After proving the presence of HPV16 DNA in the transformed cells by PCR (results not shown), Southern blot hybridization was performed with DNAs from H9, 5A, K3/I, K3/II, HEF (negative control) and CaSki cells (positive control), which had been digested with *Bam*HI. The results with ^{32}P-labelled HPV16 E6/E7 probe are shown in Fig. 1. Several bands were detected for CaSki, 5A, K3/I, K3/II and H9 cells but not for HEF cells. Distinct fragment patterns were observed with 5A and H9 cells; however, 5A cells and their K3/I and K3/II progenies exhibited similar patterns. These results indicated that HPV16 DNA had been integrated into the host DNA.

HPV16 E6/E7 transcripts in cell lines

Northern blot hybridization of total RNAs from 5A, H9, HEF and CaSki cells with ^{32}P-labelled HPV16 E6/E7 probe is shown in Fig 2. The results showed that all of the HPV16-transformed hamster cell lines expressed one transcript of similar size (approximately 2.3 kb). In addition, the extracts of H9 cells displayed three transcripts of bigger size (3.5, 4.5 and 7.2 kb). These transcripts were not present in H9/I cells derived from tumour formed in a newborn hamster by H9 cells.

*HPV16 E6*I and E6*II transcripts in cell lines*

The presence of the unspliced and spliced forms of E6 transcripts was tested by RT-PCR. In this experiment, 5A, K3/I, K3/II and two H9 cultures differing in passage level were tested. The results are shown in Fig. 3. A 420 bp unspliced E6 transcript was detected only in H9 cells (both

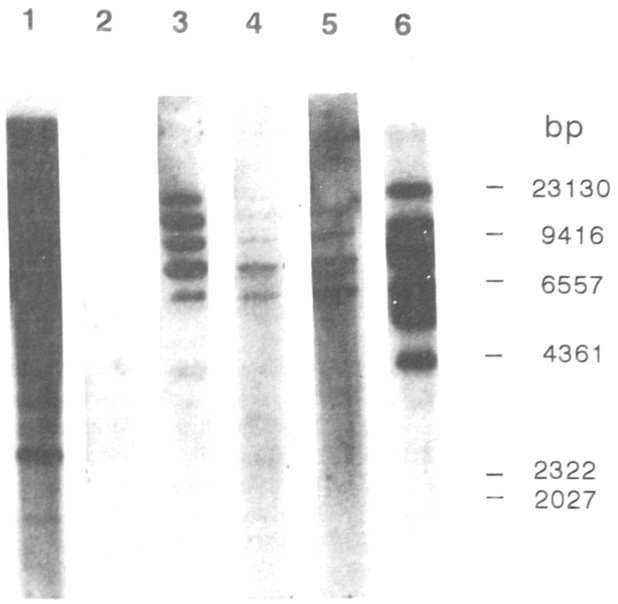

Fig. 1
Southern blot analysis: presence of HPV16 E6 DNA in transformed cell lines
CaSki, positive control (lane 1); HEF, negative control (2); 5A (3); K3/I, (4); K3/II,(5); H9, (6).

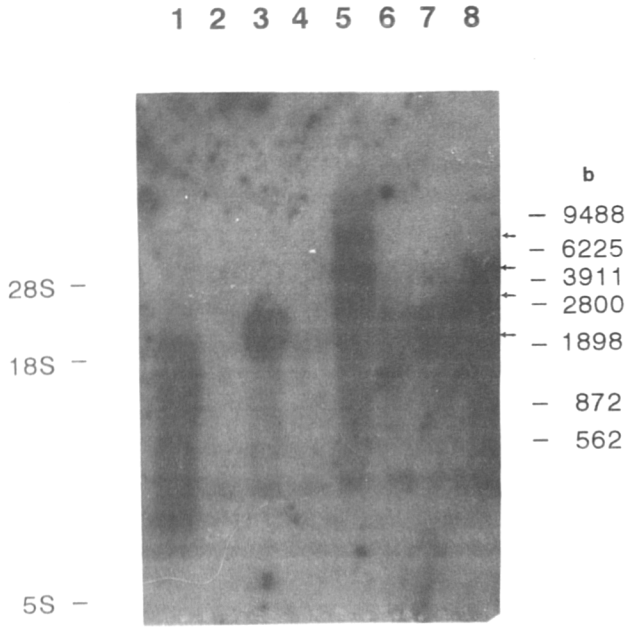

Fig. 2
Northern blot analysis of HPV16 transcripts in transformed cell lines
CaSki, positive control (lanc 1); HEF, negative control (2); H9/II (3); H9/I (4); H9 (5); K3/II (6); K3/I (7); 5A (8).

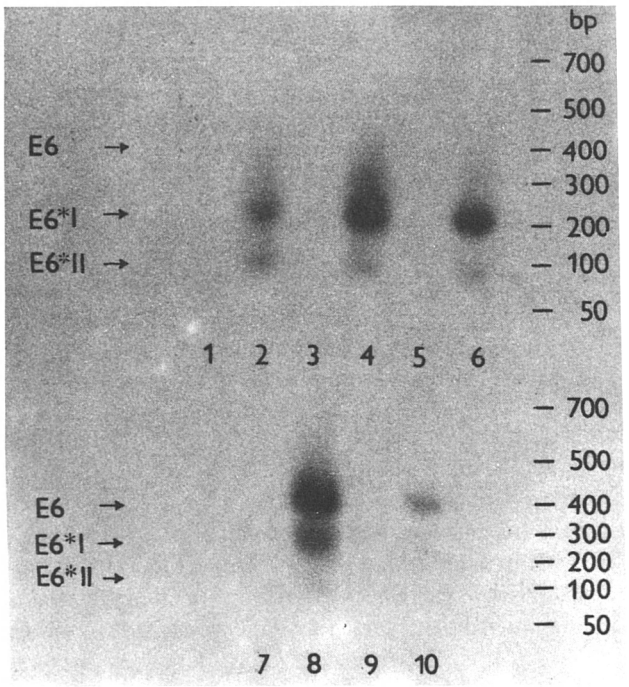

Fig. 3
Southern blot analysis of RT-PCR products derived from HPV16 E6 transcripts in transformed cell lines
K3/II (lane 2); K3/I (4); 5A (6); H9-high level passage (8); H9-low level passage (10). DNAse-treated, RT-untreated preparations were also subjected to PCR: K3/II (lane 1); K3/I (3); 5A (5); H9-high level passage (7); H9-low level passage (9).

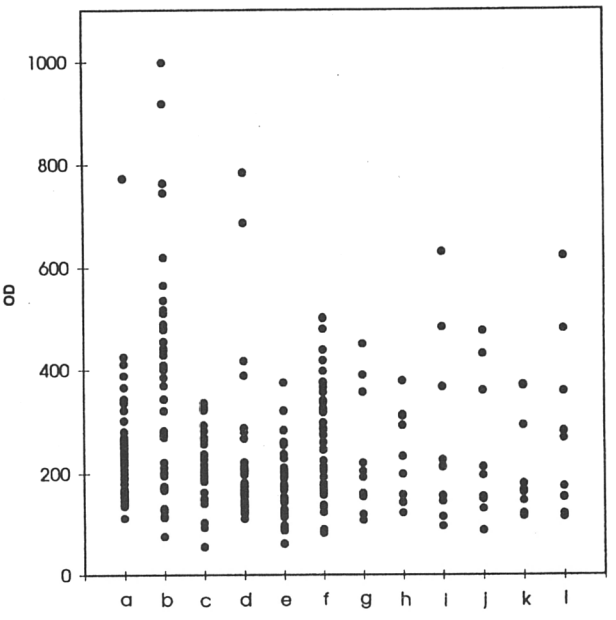

Fig. 4
Reactivity of hamster sera with HPV16 E6- and E7-derived peptides
Sera from hamsters after inoculation with K3/II cells (lanes a-f) and control hamsters (g-l) tested with E6/2 (a,g) E6/8 (b,h), E6/10 (c,i), E7/1 (d,j), E7/2 (e,k) and E7/3 (f,l) peptides.

passage levels, lanes 8 and 10). The 238 bp E6*I and 121 bp E6*II spliced transcripts were detected in 5A, K3/I, K3/II and the two H9 cell lines (lanes 2, 4, 6, 8 and 10). Interestingly, the ratio of E6*II to E6*I transcripts in 5A seemed to be different from K3/II cells. No bands were detected in control preparations not subjected to the RT reaction prior to PCR (lanes 1, 3, 5, 7 and 9) ruling out contamination by DNA. Further experiments indicated that in the tumour-derived H9/I cells the unspliced transcript was not present (results not shown).

Serological tests

Results of antibody tests with sera from 88 tumour-bearing hamsters and 13 control animals are summarized in Fig. 4. The antibody responses, if any, were weak. Only in the cases of E6/8 and E7/1 peptides, the absorbance values in a few tumour-bearing animals exceeded those detected in control animals. There was no relationship between the degree of reactivity and tumour size, cell dose administered, or either the length of time between inoculation or tumour appearance and bleeding (results not shown).

Cell-immunity tests

The results of lymphoproliferation assays with spleen cells of the hamster bearing K3/II induced tumours are shown in Table 2. Data from two experiments with the total of 18 animals are given. Findings obtained with H9 extract, which are considered significant (see Material and Methods and footnote to Table 2), are underlined. It can be seen that 11 (61%) of the animals were reactive with at least one of the antigens used. There seems to be relatively good correspondence between reactivity with the E6 and E7 peptides on one hand and the H9 reactivity on the other. Out of eight H9-reactive animals, six were also reactive with one or both peptide mixtures, and only two of nine peptide-reactive animals failed to react with H9 extract. As for reactivity with the two peptide mixtures, five animals were reactive with both E6 and E7, three were reactive with E6 only, one with E7 only and nine did not react with either E6 or E7. Thus, concordant results with the two peptide mixtures were obtained in 14 (78%) of the animals. There was no clear relationship between the reactivity and tumour size, although it may be of interest that three of four animals with the biggest tumours (tumour diameter equal to or exceeding 4.0 cm) failed to react with any of the antigens used. None of the eight control animals was reactive.

Table 2. Lymphoproliferation of spleen cells from animals bearing K3/II- induced tumors in the presence of E6- and E7-derived peptides and extracts from H9 and HEF cells

Exp. No.	Animal No.	Tumour size (cm)	Day after inoculation	SI in the presence of			
				E7 peptide mixture	E6 peptide mixture	H9	HEF
1	1	3.5	28	2.37	5.66	2.59	1.53
	2	3.5	28	–	2.44	(2.02)	1.60
	3	2.5	28	2.02	2.13	(2.29)	1.69
	4	2.5	28	–	–	2.91	0.95
	5	2.5	28	–	–	–	–
	6	2.0	28	–	–	–	–
	7	2.0	28	7.09	7.65	4.12	2.08
	8	2.0	28	–	4.67	10.65	1.08
	9	2.0	34	–	–	–	–
	10	3.0	34	–	–	(4.14)	3.65
	11	3.5	34	–	4.82	3.87	0.74
2	1	1.5	41	–	–	4.55	0.74
	2	2.5	41	2.2	4.18	9.32	3.74
	3	4.0	41	–	–	–	–
	4	4.0	41	2.26	–	2.09	1.08
	5	3.5	41	2.76	17.81	(1.99)	1.33
	6	5.0	41	–	–	–	
	7	4.0	41	–	–	–	

In some animals in which SI in the presence of H9 cell extract exceeded 2.0, SI in the presence of HEF extract was higher than 1.0. Therefore, the respective HEF-SI values are also indicated. As a positive reaction (H9-SI value underlined) only H9-SI exceeding HEF-SI by 1.0 or more was considered. SI values considered non-significant are presented in brackets.
(–)SI less than 2.0 (negative result).

Discussion

In this report we describe the establishment and some properties of lines of Syrian hamster cells transformed after transfection with HPV16 E6/E7 and Ha-ras carrying plasmids. Although the original cell lines H9 and 5A were capable of inducing tumours only in newborn and 5-day-old hamsters, cell lines were isolated from the tumours formed that were oncogenic for adult animals. The transformed cells contained integrated HPV16 DNA and viral RNA which was detected both by Northern blot hybridization and RT-PCR. Both Southern and Northern blot hybridizations as well as hybridization with the RT-PCR products revealed differences between 5A and H9 cell lines. The latter analysis demonstrated the presence of both spliced and unspliced E6 transcripts in H9 cells. On the other hand, only spliced forms of E6 transcript were demonstrated in 5A cells and their oncogenic sublines (K3/I and K3/II). Some differences were also observed in the transcription pattern between the original cell lines and their highly oncogenic progenies. The ratio between E6*I and E6*II transcripts was different in 5A cells from their more oncogenic K3/I and K3/II sublines, and the unspliced E6 transcript present in H9 cells was not detected in the tumour-derived H9/I cells. These observations suggest that the different transcript patterns may be related to the different behaviour of these cells *in vivo*, however, more quantitative studies are needed to define these differences more precisely.

In this paper we also present the first data obtained in investigating the antibody response and cell-mediated immunity in hamsters bearing tumours induced by HPV16-transformed cells. We were surprised to find that animals with tumours of different size exhibited very low reactivity with peptides known from previous studies to be reactive with sera from a significant proportion of cervical cancer patients (Dillner, 1990; Galloway, 1992; Müller *et al.*, 1992; Viscidi *et al.*, 1993; Hamšíková *et al.*, 1994). It had also been demonstrated previously that a high proportion of hamsters carrying tumours induced by other oncogenic DNA viruses, such as SV40 or adenoviruses, developed antibodies against the respective T antigens (Kitahara *et al.*, 1966; Tevethia, 1967; Vonka *et al.*, 1969). In those tests, not peptides but native proteins and not ELISA but other assays had been employed, but this should not have been of decisive importance in the context of the present knowledge. Whether our failure to demonstrate E6 and E7 antibodies in hamsters bearing HPV16-induced tumours was associated with failure of Syrian hamsters to recognize the continuous B cell

epitopes present in the peptides used, the serological assay used, the low expression of E6 and E7 proteins, their processing and/or presentation to the immune system, or possibly with the fact that the development of E6 and E7 antibodies is a late event, is not understood at this writing. It is also possible to speculate that the antibodies formed were either absorbed by the viral proteins expressed by the tumour cells or blocked by viral proteins released from the tumours into the blood stream; however, no data are available supporting this explanation. Other serological assays utilizing more complex antigens may provide more meaningful information. However, it is also possible to view the discrepancy between the findings in human and hamster sera from a different angle and assume that the development of antibodies to the E7 and E6 linear epitopes present in sera of cervical cancer patients was conditioned by extensive virus replication preceding or accompanying the development of cancer; this was not the case of HPV 16-induced tumours in hamsters in the present experiments. At variance with the serological data, a majority of tumour-bearing animals gave a positive reaction in the lymphoproliferation assay, suggesting that they recognized the T cell epitopes of E6 and E7 proteins. These findings must be interpreted with caution, however, because some of the positive results were on the brink of significance and no consistent pattern was apparent. On the other hand, the relatively good correspondence between the results obtained with the two peptide mixtures and the H9 cell extract supports the view that the reactivity monitored possessed at least a certain degree of specificity.

Acknowledgement. This work was supported by grant No. 312/93/0972 from the Grant Agency of Czech Republic and by grant No. 1914-3 from the Internal Grant Agency of the Ministry of Health of Czech Republic.

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