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Detection of herpes simplex virus type-2 DNA restriction fragments in human cervical carcinoma tissue

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DNA extracted from eight human cervical carcinomas, one lymph node metastasis and related control tissue was examined for the presence of herpes simplex virus (HSV) DNA sequences. Southern blot transfers of tumour and control DNA were hybridised with radioactively labelled cloned probes representing 70% of the HSV-2 genome. Specific hybridisation to HSV DNA sequences was observed in one of eight carcinoma tissues analysed. Hybridisation of HSV-2 DNA probes to *Bam*HI and *Xho*I restriction enzyme fragments of tumour cell DNA which co-migrated with authentic HSV-2 viral fragments identified co-linear HSV-2 DNA sequences comprising 3% of the HSV-2 genome, between map coordinates 0.582 and 0.612. The remaining eight tumour and all control tissues analysed, showed no specific hybridisation to any of the probes used at levels of sensitivity which would detect 0.5 copies/cell of HSV-2 DNA restriction fragments of 2 kb or greater.

Key words: cervical carcinoma/HSV-2 DNA/Southern transfer

Introduction

Initial cytological observations showing a relationship between genital herpes infection and cervical neoplasia (Naib *et al.*, 1966) stimulated several large seroepidemiological surveys, in which higher mean antibody titres to herpes simplex virus type-2 (HV-2) were detected in women with carcinoma of the cervix than in matched controls (Rawls *et al.* 1969; Adam *et al.*, 1973; Gilman *et al.*, 1980).

Epidemiological studies had previously proposed that venereally transmitted factors were involved in the development of uterine cervical cancer (Kessler, 1976), and the increasing prevalence of HSV-2 as a sexually transmitted agent has stimulated attempts to establish an association between previous infection with HSV-2 and development of uterine cervical cancer.

HSV-specific antigens are detected in cervical carcinoma cells by immunofluorescence tests (Royston and Aurelian, 1970; Nahmias *et al.*, 1975) and recent studies using monospecific antisera have demonstrated HSV-2 DNA binding proteins ICSP 11/12, and ICSP 34/35 in eight out of 21 cervical carcinoma tissue sections analysed (Dreesman *et al.*, 1980; McDougall *et al.*, 1982). There has been only one report of HSV DNA in tumour material (Frenkel *et al.*, 1972) and subsequent extensive studies have failed to detect viral DNA, (zur Hausen *et al.*, 1974; Pagano, 1975). However, RNA transcripts homologous to a limited portion of the HSV genome were demonstrated in cervical carcinoma sections from *in situ* hybridisation studies (Jones *et al.*, 1978; McDougall *et al.*, 1980; Eglin *et al.*, 1981a). Transcripts homologous to HSV-2 DNA probes from 0.07 to 0.40 genome map units (mu) and from sequences bounding the short unique region of the genome (0.85–0.87 mu, 0.88–0.98 mu) were detected in up to 60% of tumour sections analysed (Eglin *et al.*, 1981b) and an additional region from 0.58 to 0.63 mu was detected in up to 10% of invasive carcinoma tissue sections by McDougall *et al.* (1982).

HSV-1 and HSV-2 are capable of transforming cells in culture. Two regions have been implicated in morphological transformation by HSV-2, these map between 0.41 and 0.58 mu (*Bgl*II *c*; Jariwalla *et al.*, 1980) and 0.58–0.62 mu (morphological transforming region 2, MTR2; Reyes *et al.*, 1979; Galloway and McDougall, 1981; Cameron and Macnab, 1980). However, a single region has been implicated in morphological transformation by HSV-1 (MTR1 0.31–0.41 mu, Camacho and Spear, 1978; Reyes *et al.*, 1979). The significance of this difference between these two otherwise closely related viruses is not understood. Transcripts detected in carcinoma sections therefore correspond to two (MTR1 and MTR2) of the three regions implicated in morphological transformation by HSV-1 and HSV-2. Failure to detect transcription from other regions of the genome suggests that only a portion of HSV DNA is retained within neoplastic cells, or that there is restricted expression of the viral sequences present. Previous attempts to demonstrate HSV DNA in cervical tissue (zur Hausen *et al.*, 1974; Pagano, 1975) have probably failed as a result of the use of techniques which, at their limit of sensitivity, would be unable to detect sub-genomic fragments of viral DNA present at low copy number, and the experience with other DNA transforming viruses suggests that as little as 1.5–3% of the HSV genome could contain all information required for transformation (Gallimore *et al.*, 1974; Novak *et al.*, 1980; Martin, 1981).

The demonstration of HSV RNA and antigens in cervical carcinoma tissue has stimulated us to examine human cervical carcinoma tissue further for the presence of HSV-2 DNA, by hybridisation of cloned HSV-2 DNA fragment probes to Southern blot transfers (Southern, 1975) of restriction enzyme-digested tumour DNA. In reconstruction experiments, this technique routinely detected HSV-2 DNA restriction fragments equivalent in size to 1.5% of the viral genome at a level of 0.5 copies/cell. Here we report the presence of HSV-2 DNA sequences from a region comprising 3% of the viral genome (0.58–0.612 mu) in one out of eight carcinoma tissues analysed.

Results

Cervical carcinoma and control tissue

Eight human cervical carcinomas and a number of related tissues have been examined; these included a lymph node metastasis from one of these tumours (CaCx126), a cell line established from an explanted human cervical squamous cell carcinoma (CaCx106), (Kitchener, in preparation) and a tumour derived from this cell line in a nude mouse. Seven of the tumours analysed were squamous cell carcinomas of the cervix and one (CaCx122) was an adenocarcinoma of the cervix.

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Two types of control tissue associated with potential HSV infection were analysed. This included DNA extracted from nine colposcopically directed punch biopsies taken from the squamocolumnar junction (transformation zone), of normal cervices, since cervical neoplasia usually arises from this region (Stern, 1973) and a biopsy of human adult brain. DNA was also extracted for analysis from tissues in which previous herpesvirus infection would not be expected. These included two specimens of human foetal tissue, a human ovarian cancer and two established human embryo lung cell lines (Helu and MRC5).

Ectocervical swabs taken prior to biopsy or operation were all negative when tested in tissue cultures for herpesvirus infection, and tissue explants either alone or after co-cultivation with permissive cells failed to yield infectious HSV.

Analysis of tumour and control DNA for HSV-2 sequences

Cervical carcinoma and control tissue DNA was examined for the presence of HSV DNA using the conventional blotting and DNA/DNA hybridisation technique of Southern (1975). The cloned restriction enzyme fragment probes employed (Figure 1) represented ~70% of the HSV-2 genome and were selected to include those viral sequences previously detected in carcinoma tissue by *in situ* hybridisation and associated with morphological transformation of rodent cells *in vitro* (MTR1, MTR2 and *Bgl*II *c*, Figure 1). All cloned HSV-2 fragments were excised from vector sequences and purified twice by agarose gel electrophoresis (rigorous purification was necessary to eliminate hybridisation of vector sequences to several human tumour DNAs) prior to labelling *in vitro* with [α-^{32}P]CTP and [α-^{32}P]GTP to specific activities of $4 \times 10^7 - 4 \times 10^8$ c.p.m./μg DNA by nick-translation (Rigby *et al.*, 1977).

Tumour and control tissue DNAs digested with *Bam*HI were hybridised with the *Eco*RI *j*, *Hind*III *h*, *Hind*III *e*, *Bgl*II *n*, and *Bam*HI *s* probes shown in Figure 1. Hybridisation was observed with only one probe (*Bgl*II *n*), to only one tumour DNA (CaCx122, Figure 2B). This revealed a novel 9.8-kb fragment and a 2.8-kb fragment which co-migrated with the *Bam*HI *t* fragment of HSV-2 (Figure 2B). These HSV sequences were contained within one fragment of 13.5 kb liberated after *Hind*III digestion of CaCx122 DNA to which the *Bgl*II *n* (Figure 2A) or a *Hind*III *a* (data not shown) probe hybridised. All other tumour tissues or control tissues were negative for hybridisation at levels of sensitivity, with each probe used, which would detect 0.5 copies/cell of an HSV-2 DNA restriction fragment of 2 kb or greater.

Sequence arrangement in CaCx122

The viral specificity of the 2.8-kb *Bam*HI fragment detected in CaCx122 DNA with the *Bgl*II *n* probe (Figure 2B), was confirmed when CaCx122 DNA was digested with restriction endonuclease *Xho*I and hybridised with a *Bam*HI *t* probe (0.584 – 0.602) (Figures 1 and 3). Digestion of HSV-2 DNA with *Xho*I produces two fragments of 2.4 kb and 2.0 kb containing *Bam*HI *t* specific sequences (Figure 4, fragments 2 and 3, respectively). Hybridisation of *Xho*I-digested CaCx122 with the *Bam*HI *t* probe revealed that fragments 2 and 3 were present in their entirety (Figure 3). Positive hybridisation of *Bam*HI *a* probe to *Xho*I fragment 2 and of *Bam*HI *e* probe to *Xho*I fragment 3 (Figures 3 and 4) confirms the viral specificity of these sequences. Probe *Bam*HI *e* did not hybridise to the *Xho*I fragments 4, 5, 7, 8 and 9 (Figures 3 and 4) suggesting that the HSV sequences do

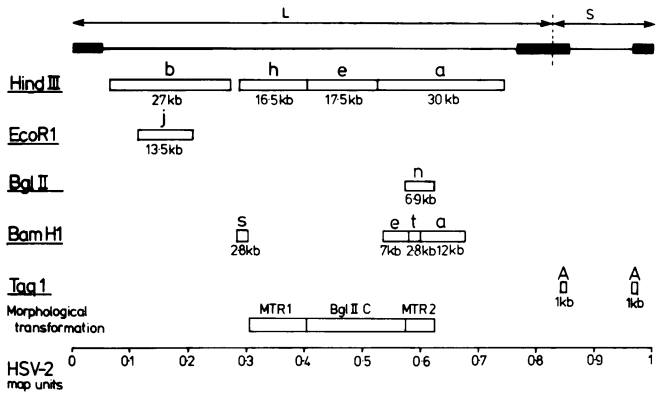

Fig. 1. Diagram of the HSV-2 genome showing the location of the cloned restriction fragments used as probes in blot hybridisations (as described in Materials and methods). The complete HSV genome is ~150 kb; solid boxes represent terminal and internal repeat segments which bound both the long (L) and short (S) unique components of the HSV genome, (Sheldrick and Berthelot, 1974). DNA fragments associated with transformation *in vitro* by HSV-1 (MTR1) and HSV-2 (MTR2, *Bgl*II *c*) are as described in the text.

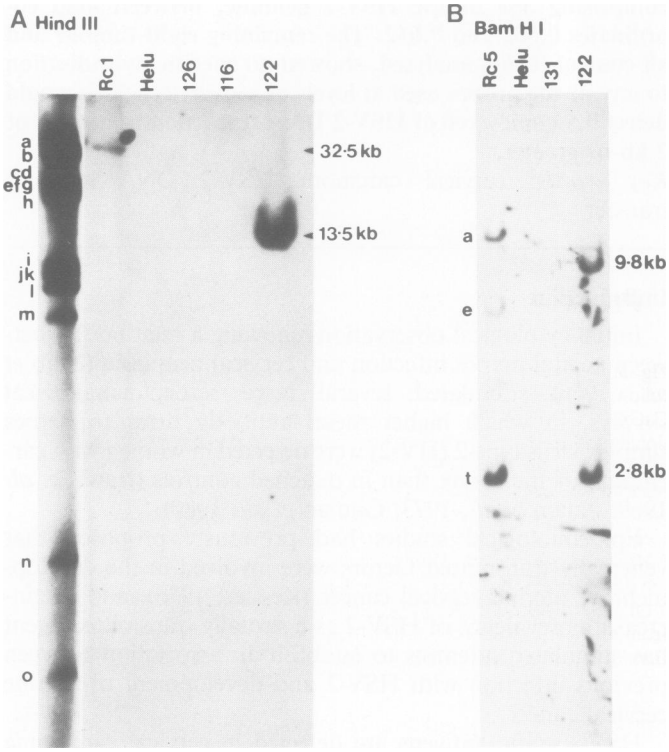

Fig. 2. Detection of fragments of HSV-2 DNA in DNA from cervical tumours (nos. 116, 126, 122, 131), 20 μg of high mol. wt. DNA extracted from tumours and cell lines was digested with an excess of *Hind*III (**A**) or *Bam*HI (**B**). The products of digestion were fractionated by electrophoresis through 0.6% agarose slab gels, transferred to nitrocellulose membrane by the procedure of Southern (1975) and hybridised to a ^{32}P-labelled *Bgl*II *n* probe excised from the HSV-2 cloned *Hind*III *a* fragment; then exposed for autoradiography with Dupont Lightning Plus intensifier screens for 2 – 5 days. Lanes RC1 and RC5 (**A** and **B**) contain a reconstruction with 1 copy (0.5 ng) and 5 copies (2.5 ng) respectively, of HSV-2 DNA, which has been added to 20 μg of human embryo lung (Helu) DNA and treated as described above. *Hind*III digests (left track, **A**) of HSV-2 DNA provided mol. wt. markers in both gels (track in **B** is not shown). The *Bgl*II *n* probe hybridises to one HSV-2 DNA band after *Hind*III digestion (*Hind*III *a* 32.5 kb **A** and Figure 1) and three HSV-2 DNA bands after *Bam*HI digestion (*Bam*HI *a, e, t*,**B** and Figure 1).

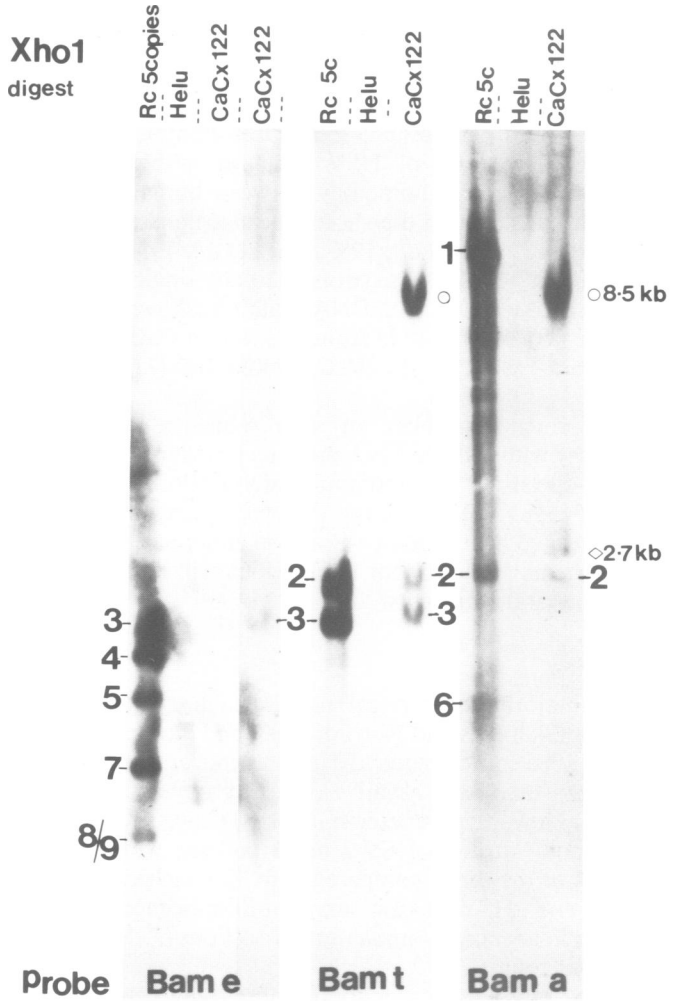

Fig. 3. Detection of fragments of DNA containing HSV-2 DNA sequences following cleavage of CaCx122 DNA with *Xho*I. Triplicate samples of CaCx122 and Helu DNA (20 μg each) were digested with *Xho*I, fractionated through an 0.7% agarose gel and treated as Figure 2. The nitrocellulose filter was divided into three and each strip hybridised to separate ^{32}P-labelled HSV-2 *Bam*HI probes, *Bam*HI *e, t, a* (see Figures 1 and 4) allowing alignment of common bands after autoradiography. A reconstruction track with five copies of HSV-2 DNA (Rc5) plus 20 μg Helu DNA and digested with *Xho*I is present on each strip, fragment numbers are diagrammatically represented in Figure 4. Faint bands below fragment 3 on reconstruction track (Rc5) when hybridised to *Bam*HI *t* may result from low levels of exonuclease activity present in the enzyme preparation. *Bam*HI-digested HSV-2 DNA provided mol. wt. markers (not shown).

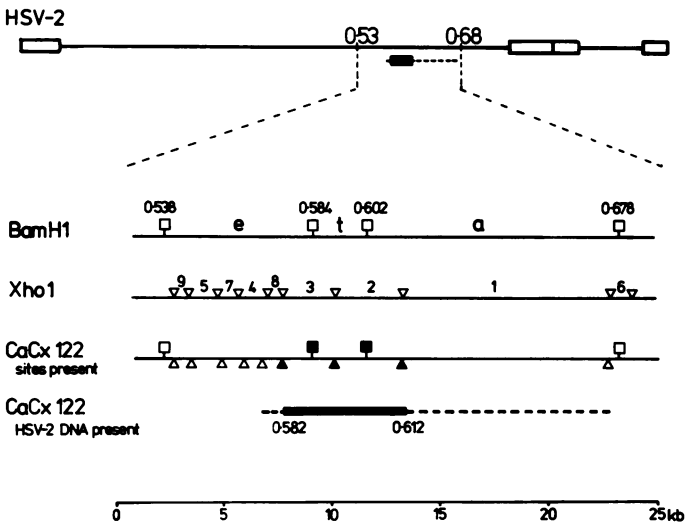

Fig. 4. HSV-2 DNA sequences present within CaCx122 DNA. HSV-2 *Bam*HI sites and map coordinates are marked □, *Xho*I sites within this region are depicted ∇. Those restriction sites present within CaCx122 DNA are represented by solid symbols and those not detected are represented by open symbols. The HSV-2 DNA sequences present are depicted by the solid line between map coordinates 0.582 and 0.612 and those regions of uncertainty are represented by a dashed line. The HSV-2 DNA in CaCx122 cannot be represented by a simple model and requires further characterisation before an accurate map of HSV sequences present can be constructed.

not extend far beyond the *Xho*I site at 0.582 map units. The novel fragment of 2.7 kb (Figure 3) detected with *Bam*HI *a* probe may represent a junction between host and viral DNA sequences. Both the *Bam*HI *a* and *Bam*HI *t* probes also hybridise strongly to an *Xho*I fragment of 8.5 kb (Figure 3) and the simplest explanation for this, and the loss of the expected *Xho*I site at 0.612 map units (mu), would be that additional copies of these viral sequences are also present in CaCx122 in a rearranged form. Rearrangement of integrated viral DNA sequences has been detected in SV40- and adenovirus-transformed and tumour cell DNA (Clayton and Rigby, 1981; Eick and Doerfler, 1982).

The hybridisable DNA detected in CaCx122 can, therefore, mostly be identified as HSV-2 specific and consists in part of viral sequences co-linear with the HSV-2 genome between map co-ordinates 0.582 and 0.612 (Figure 4), at ~3–5 copies/cell by comparision with the reconstruction tracks (Figures 2B and 3). Sequences within the *Hind*III *a* probe from 0.52 to 0.54 mu and from 0.69 to 0.74 mu are not represented in either the *Bgl*II *n* or *Bam*HI *a, t* or *e* probes and hybridisation of probes from these regions of the HSV-2 genome to CaCx122 tumour DNA cannot be discounted. However, this seems unlikely since the *Bam*HI *e* probe did not hybridise to *Xho*I fragments 4, 5, 7, 8 and 9 (Figures 3 and 4) between 0.54 to 0.58 mu and the *Bam*HI *a* probe did not hybridise to *Xho*I fragment 6 (Figures 3 and 4).

Tumour tissue consists of a mixed population of cells and it is therefore possible that HSV-2 sequences are present in only a subpopulation of cells in the tumour but at relatively high copy number. The identification of only one fragment of 13.5 kb in *Hind*III-digested CaCx122 DNA to which the *Hind*III *a* (data not shown) or *Bgl*II *n* (Figure 2A) probes hybridised suggests that the HSV-2 DNA identified may be present in a reiterated or amplified form. Small stretches of HSV-2 DNA sequences may not, however, be detected in host/viral junction fragments. The HSV-2 DNA in CaCx122 therefore cannot be represented by a simple model at this stage, and the use of smaller HSV-2 DNA probes and a variety of restriction enzymes will reveal the precise structure.

Limits of sensitivity for negative hybridisation results; cross-hybridisation between human and HSV DNA sequences

The results from *in situ* hybridisation (Eglin *et al.*, 1981b; McDougall *et al.*, 1982) strongly suggested that positive hybridisation would be expected with HSV-2 DNA probes selected from 0.07 to 0.40 mu and with probes selected from sequences bounding the short unique region of the genome.

The HSV-2 DNA region represented by the *Hind*III *h* probe (Figure 1, 0.29–0.40 mu) frequently shows hybridisation to cervical carcinoma tissues *in situ* and codes for the HSV-2 DNA binding protein ICSP 11/12 detected in cervical carcinoma sections (Dreesman *et al.*, 1980; McDougall *et al.*,

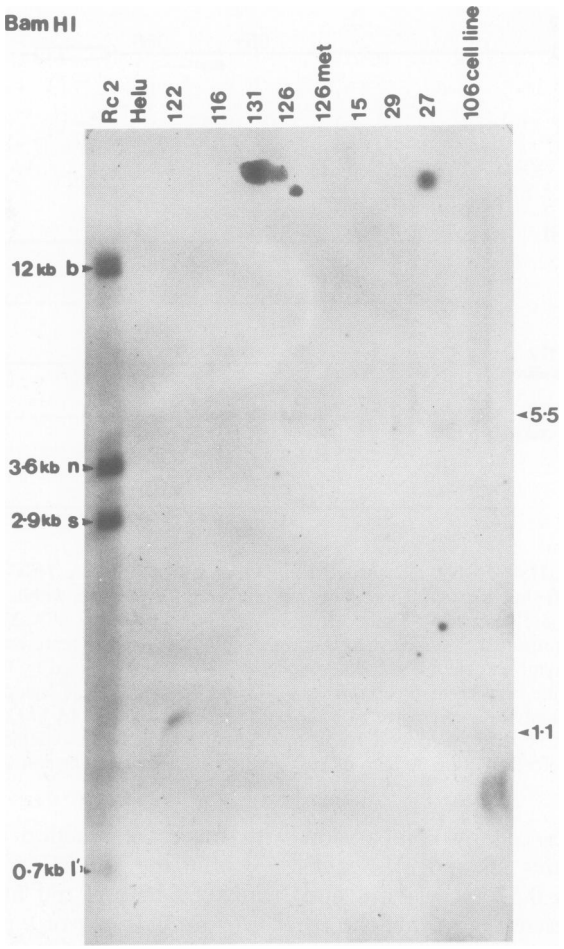

Fig. 5. Hybridisation of *Hind*III *h* probe (Figure 1) to DNA from cervical tumours 122, 116, 131, 126, 126 metastases, 15, 27, 29 and DNA extracted from cell line CaCx106 established from a human cervical tumour. High mol. wt. DNA (20 μg) from tumours and cell lines was digested with an excess of *Bam*HI, fractionated through a 0.6% agarose gel and treated as Figure 2. Lane Rc2 contains a reconstruction with two copies (1 ng) of HSV-2 DNA, which has been added to 20 μg of Helu DNA and treated as above. *Bam*HI-digested HSV-2 DNA provided mol. wt. markers (not shown). The *Hind*III *h* probe hybridises to five HSV-2 DNA fragments after *Bam*HI digestion (*Bam*HI, *b, n, s, l'* and *n'* which is not resolved).

1982). The *Hind*III *h* probe (Figure 5) hybridises weakly to two fragments of 5.5 kb and 1.1 kb present in all *Bam*HI-digested human tumour and normal DNAs analysed, which presumably represent cellular sequences. However, at the level of sensitivity attained, this probe failed to hybridise to any apparent HSV sequences. It is clear that the experiment was capable of detecting 0.5 copies/cell of an HSV DNA fragment equivalent to or larger than the size of *Bam*HI *s* (2.85 kb, Figure 5) but would not detect small HSV fragments at this level (the HSV-2 *l'* fragment 0.7 kb in the reconstruction track in Figure 5 is barely visible using this probe). The *Hind*III *e*, *Eco*RI *j* and *Bam*HI *s* probes (Figure 1) also show no hybridisation to any herpesvirus-specific sequences at a similar level of sensitivity, nor do they hybridise to any cellular DNA sequences (data not shown).

The HSV-1 *Taq*I *A* fragment (Figure 1) selected from the short repeat region of the HSV genome which contains an origin of HSV DNA replication (Stow, 1982) showed a diffuse hybridisation pattern to all human DNAs analysed, but also hybridised to several common cellular fragments of 4.5 kb, 1.8 kb and 1.3 kb after *Bam*HI digestion (data not shown). This is not unexpected since this probe contains several short repeated sequences (Watson *et al.*, 1981; Murchie and McGeoch, 1982) which may share homology with some dispersed repetitive cellular sequences. Maitland *et al.* (1981) showed that cellular sequences homologous to the short repeat regions of HSV were transcribed in human placental tissue and homology between human DNA and HSV, in particular to the short repeat regions of HSV, has been shown (Peden *et al.*, 1982; Puga *et al.*, 1982). The *Taq*I *A* probe did not hybridise strongly to any single fragment in *Bam*HI-digested CaCx122 DNA (data not shown) suggesting that the HSV hybridisable sequences within CaCx122 do not arise from HSV class I-defective molecules (Frenkel *et al.*, 1980).

The presence in HSV of such sequences which share homology with cellular DNA presents problems for detection of HSV-specific DNA in tumour material. Positive identification of HSV DNA must therefore depend upon the demonstrated liberation by restriction endonuclease digestion of fragments from tumour DNA which will co-migrate with authentic viral fragments.

Discussion

The majority of cervical neoplasias are squamous cell tumours (Nahmias and Norrild, 1980) and have consequently been studied more frequently than adenocarcinomas, which have been used as a negative control group by Eglin *et al.* (1981b). However, Wenczer *et al.* (1981) have shown increased antibody titres to HSV-2 in 16 patients with adenocarcinomas of the cervix compared with 32 matched control patients, and HSV-specific immunofluorescence has been detected in adenocarcinoma tumour sections (J. Macnab, unpublished results).

HSV-2 DNA sequences found most frequently in cervical carcinoma tissue sections from the *in situ* hybridisation results of Eglin *et al.* (1981b) are those from 0.07 to 0.40 mu in the long unique region and sequences bounding the short unique region of the genome, whereas those HSV-2 DNA sequences identified in CaCx122 correspond to the MTR2 region of HSV-2. Sections of this tumour were not assayed by *in situ* hybridisation and therefore it is not known whether transcripts would have been detected from either region. McDougall *et al.* (1982) have reported that 10% of cervical carcinoma sections analysed by *in situ* hybridisation are also positive for the MTR2 region. However, immunoperoxidase staining assays (McDougall *et al.*, 1982) using a monoclonal antiserum to a 38-K HSV-2 polypeptide known to be encoded by this region (Docherty *et al.*, 1981; Galloway *et al.*, 1982) failed to identify this protein in cervical carcinoma sections, where the HSV-2 major DNA binding protein ICPS 11/12 mapping between 0.38 and 0.41 mu (Morse *et al.*, 1978; Marsden *et al.*, 1978) could be detected. Sera from patients with cervical carcinoma, however, will preferentially immunopreciptate two HSV-2 polypeptides of 118 K and 38 K (which may represent the 38-K polypeptide encoded in MTR2, Docherty *et al.*, 1981; Galloway *et al.*, 1982).

The identification of authentic viral sequences in only one out of eight cervical tumours is in contrast to published *in situ* hybridisation results where 60% of cervical carcinoma sections analysed can show positive hybridisation with cloned HSV-2 DNA probes. The use of *in situ* hybridisation of DNA probes to RNA provides a very sensitive technique capable of detecting transcripts in isolated cells, the importance of which

is evident since tumours may consist of both apparently normal and neoplastic cells. The large numbers of negatives found in this sample and experienced by others (zur Hausen *et al.*, 1974; Pagano, 1975) presumably reflect the insensitivity of the technique used, which at its limit in this study would detect an HSV-2 DNA fragment ≥1.5% of the viral genome at 0.5 copies/cell. This may be a reflection of the large size of many of the probes used, e.g., *Hind*III *h, e, a* and *Eco*RI *j* (depicted in Figure 1) range in size from 13.5 to 32.5 kb; however, by comparison with the respective reconstruction tracks, these probes would detect an HSV-2 specific fragment of ~2 kb at 0.5 copies/cell and a fragment of 1 kb at 1 copy/cell. This study, therefore, does not exclude the possibility that other HSV-specific sequences are present within the tumours analysed and are undetected by this approach.

The approach of Southern blot hybridisation, however, although treating the tumour mass as a whole, has provided direct evidence for the presence of HSV-2 DNA in one cervical carcinoma tissue, the analysis of which has identified precisely the HSV-specific sequences present, providing additional information on the structural organisation of this DNA. The identification of HSV-2 DNA at 3–5 copies/cell in one cervical carcinoma which corresponds to a region of HSV-2 DNA (MTR2) previously associated with morphological transformation of rodent cells is interesting, since MTR2 DNA is not retained within transformed rodent cells at high copy number (Galloway and McDougall, 1981; Reyes *et al.*, 1979) and in several instances is undetectable by Southern blot analysis after the transformation event (Galloway and McDougall, 1983; Cameron and Macnab, unpublished results).

Our present inability to identify a single gene or common DNA sequence essential for the initiation or maintenance of transformation by HSV *in vitro* has led to the proposal of a 'hit and run' model for HSV transformation (Skinner, 1976; Hampar *et al.*, 1976; Galloway and McDougall, 1983). Since a significant proportion (40–60%) of cervical carcinoma tissue sections show no positive hybridisation *in situ* with HSV-2 DNA probes and are negative for HSV-specific antigens, there would appear to be no dependence on continued expression of HSV in these cervical tumours. The results from this study and the recent report (Galloway and McDougall, 1983) that DNA from three out of nine cervical tumours was positive by Southern blot hybridisation with HSV-2 DNA probes, lends support for involvement of HSV in cervical carcinogenesis even though retention of HSV DNA within these tumours may be fortuitous. In proposing a role for HSV in cervical carcinogenesis, HSV may act as an initiator of a transforming event through induction of heritable mutational changes or perhaps through interaction with cellular oncogenes. Additionally, HSV may interact as a co-carcinogen with other agents which infect the cervix such as papillomaviruses (zur Hausen, 1982).

Human papillomavirus (HPV 6 and HPV 11) DNA has been demonstrated in four out of 55 invasive carcinoma biopsy specimens and in premalignant lesions (Gissman *et al.*, 1982, 1983; Green *et al.*, 1982). Cervical squamous cell papillomas rarely develop into invasive carcinomas (Kazal and Lang, 1958), however, a synergistic interaction of papillomaviruses with co-carcinogens has been demonstrated for both bovine papillomaviruses (Jarrett *et al.*, 1980) and for human papillomavirus type 5 in epidermodysplasia verruciformis (Orth *et al.*, 1980).

At present, any role for HSV in cervical carcinogenesis is therefore unclear, but the ability to detect HSV DNA in one human tumour will facilitate cloning of such sequences together with any flanking cellular sequences, analysis of which may clarify any association HSV has with the development of cervical carcinoma.

Materials and methods

Plasmids

The recombinant plasmids pGz25, pGz11, pGz25 and pGz10 contain HSV-2 *Hind*III fragments *a, b, e* and *h*, respectively (Eglin *et al.*, 1981b) and were cloned into the *Hind*III site of plasmid vector pAT153 (Twigg and Sherratt, 1980). Plasmid pGx59 contains a 995-bp HSV-1 *Taq*I fragment cloned into the *Cla*I site of pAT153 and was kindly supplied by N. Stow (Stow, 1982). The HSV-2 *Bam*HI *e* and *t* fragments provided by J. McLauchlan were cloned into the *Bam*HI site of pAT153 and the *Bam*HI *a* fragment cloned into the *Bam*HI site of pBR322 was kindly supplied by A.C. Minson.

Plasmid propagation and purification were essentially as described by Davison and Wilkie (1981). All HSV restriction fragments used as probes were excised then separated from vector sequences by two cycles of agarose gel purification. *Eco*RI *j* and *Bgl*II *n* were generated as subfragments after digestion of plasmids pGz11 and pGz26, respectively.

Preparation of cell and tumour DNA

Tumour tissue was finely minced with scissors and total cell nucleic acids were isolated by proteinase K digestion (200 μg/ml in 0.4% SDS, 0.05 M Tris-HCl pH 8.0, 0.01 M EDTA, 0.1 M NaCl) and phenol extraction (Varmus *et al.*, 1973), digested with RNase A (100 μg/ml), re-extracted sequentially with phenol and chloroform, dialysed extensively against 0.15 M NaCl and 0.015 M sodium citrate, ethanol precipitated and the resulting DNA redissolved in H_2O.

Blot hybridisation

The detection of fragments of HSV-2 DNA in DNA from cervical tumours was examined using the conventional blotting and DNA/DNA hybridisation technique of Southern (1975). High mol. wt. DNA extracted from tumours or control tissue was cleaved with an excess of the restriction endonucleases, *Hind*III, *Bam*HI or *Xho*I using conditions recommended by the supplier. The products of digestion were separated by electrophoresis through 0.6% or 0.7% horizontal agarose slab gels and transferred to nitrocellulose filters (Southern, 1975). HSV-2 DNA fragments were labelled *in vitro* with [α-^{32}P]CTP and [α-^{32}P]GTP by nick-translation (Rigby *et al.*, 1977) to specific activities of 5 x 10^7–4 x 10^8 c.p.m./μg. DNA probes were denatured with alkali and hybridised for 20 h at 72°C in 3 x SSC (SSC = 0.15 M sodium chloride plus 0.015 M sodium citrate), 0.5% SDS, 50 μg/ml denatured salmon sperm DNA, 0.02% Ficol, 0.02% polyvinylpyrolidone, 0.02% bovine serum albumin and 10% dextran sulphate. The filters were then treated with decreasing stepwise washes of 3 x SSC–0.2 x SSC plus 0.1% SDS and 10 mM pyrophosphate at 72°C. Filters were air dried and exposed for autoradiography with Dupont lightning plus intensifier screens for 2–5 days.

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