Detection and characterization of human papillomavirus type 45 DNA in the cervical carcinoma cell line MS751

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The cervical carcinoma-derived cell line MS751 was examined for human papillomavirus (HPV) DNA and RNA. A genomic fragment containing both viral and cellular sequences was cloned. Sequence analysis showed that MS751 cells contain a partially deleted HPV-45 genome integrated at a single chromosomal site. HPV sequences from the E6–E7 region are expressed as poly(A) RNA.

Human papillomavirus (HPV) types are known to contribute to the development of cervical cancer. This is documented by numerous epidemiological and experimental studies (Schiffmann, 1994; zur Hausen, 1994). Cancer-associated genital HPVs are represented by types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 68 (de Villiers, 1989, 1994; Jacobs et al., 1995). HPV-16 and HPV-18 are detected more frequently in cervical carcinomas than any of the other HPV types (Bosch et al., 1995; Lörincz et al., 1992). Cell lines derived from cervical cancer are often used as model systems to analyse virus–host cell interactions which may contribute to carcinogenesis. On the basis of DNA and RNA filter hybridization data, cells of the cervical carcinoma-derived cell line MS751 were assumed to contain HPV-18 DNA (Pater & Pater, 1985; Yee et al., 1985).

In this study, we have analysed the HPV sequences in MS751 cells by molecular cloning, DNA sequencing and Northern blot hybridization. The cell line was obtained from the ATCC (HTB 34). First, MS751 genomic DNA was examined for the presence of HPV-18 sequences by Southern blot analysis. DNA was isolated from MS751 cells and C-41 cells by proteinase K treatment and phenol extraction. C-41 cells were used for control purposes since these cells are known to contain a single copy of HPV-18 DNA per cell (Schwarz et al., 1985). After restriction endonuclease digestion and electrophoretic separation in a 0.7% agarose gel, DNA was transferred to a GeneScreen nylon membrane (Dupont NEN). Cloned HPV-18 DNA was radiolabelled by the random priming method (Feinberg & Vogelstein, 1983). Hybridization was first performed under stringent conditions [50% formamide, 5 x SSC, 50 mM sodium phosphate pH 6.5, 5 x Denhardt’s solution, 0.1 mg/ml tRNA, 1% SDS and 3 x 10^6 c.p.m./ml denatured gel-purified ^32P-labelled HPV-18 DNA (> 5 x 10^8 c.p.m./µg) at 42 °C]. The filter was washed in 2 x SSC, 0.1% SDS at 68 °C and exposed for several days using intensifying screens. Under these conditions, only very faint signals were observed for MS751 DNA (data not shown). This filter was then stripped and reprobed with HPV-18 DNA under conditions of reduced stringency (30% instead of 50% formamide which corresponds to Tm – 36 °C instead of Tm – 18 °C, respectively). The filter was washed in 2 x SSC, 0.1% SDS at 50 °C (Tm – 36 °C). For each restriction digest one or several prominent bands and numerous faint bands were evident (Fig. 1a). After exposure the filter was washed under stringent conditions (Tm – 18 °C) and re-exposed for the same length of time. As expected the signal intensity of the major bands for C-41 were slightly reduced. In contrast, the signal intensities of the bands for MS751 DNA decreased considerably (Fig. 1b). These results indicate that MS751 cells do not contain HPV-18 DNA but rather the DNA of a papillomavirus related to HPV-18.

For molecular cloning of the HPV-18-related papillomavirus DNA, genomic MS751 DNA was cleaved with EcoRI. Digestion with this restriction enzyme produces a single fragment of about 9.5 kb in length that hybridized to the HPV-18 probe (Fig. 1b). To enrich for virus-specific DNA, EcoRI-cleaved MS751 DNA was fractionated in a 0.7% agarose gel and DNA fragments of about 9.5 kb were eluted. For construction of a phage library, EcoRI-cleaved, dephosphorylated LambdaGEM-12 arms (Promega) were used. Lambda arms and genomic inserts were ligated using equal molar ratios. In vitro packaging of the DNA was done with Packagene extracts from Promega. Recombinant phages were amplified in E. coli strain LE392. Plaque hybridization of replica filters was performed under conditions of reduced stringency (Tm – 36 °C) with two HPV-18 subgenomic probes (1.8 kb and 3.4 kb). This approach was chosen to distinguish more easily between positive and false-positive clones. The 1.8 kb fragment comprises part of the E7-ORF and E1-ORF (nucleotide positions 658–2472), the 3.4 kb fragment comprises part...
Fig. 1. Southern blot analysis of MS751 and C-4 I DNA with HPV-18 as hybridization probe. (a) Hybridization was performed under low-stringency conditions ($T_m = 36 \, ^\circ C$). (b) Same filter as in (a) washed under stringent conditions ($T_m = 18 \, ^\circ C$) and re-exposed under identical conditions. The arrowheads indicate the positions (bp) of the lambda HindIII size-marker fragments. As sensitivity markers 100 pg and 10 pg of cloned HPV-18 DNA were used.

Table 1. Summary of all nucleotide exchanges and amino acid substitutions after sequence alignment of the E6- and E7-ORF of HPV-45 from MS751 and that of the reference clone

<table>
<thead>
<tr>
<th>ORF</th>
<th>Nucleotide position</th>
<th>Base exchange</th>
<th>Amino acid substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>E6</td>
<td>259</td>
<td>A $\leftrightarrow$ G</td>
<td>Tyrosine $\leftrightarrow$ cysteine</td>
</tr>
<tr>
<td>E6</td>
<td>497</td>
<td>G $\leftrightarrow$ A</td>
<td>Glycine</td>
</tr>
<tr>
<td>E7</td>
<td>590</td>
<td>T $\leftrightarrow$ C</td>
<td>Tyrosine $\leftrightarrow$ histidine</td>
</tr>
<tr>
<td>E7</td>
<td>600</td>
<td>A $\leftrightarrow$ G</td>
<td>Glutamine $\leftrightarrow$ arginine</td>
</tr>
<tr>
<td>E7</td>
<td>603</td>
<td>C $\leftrightarrow$ A</td>
<td>Alanine $\leftrightarrow$ glutamic acid</td>
</tr>
<tr>
<td>E7</td>
<td>670</td>
<td>C $\leftrightarrow$ T</td>
<td>Cysteine</td>
</tr>
<tr>
<td>E7</td>
<td>769</td>
<td>C $\leftrightarrow$ A</td>
<td>Asparagine $\leftrightarrow$ lysine</td>
</tr>
<tr>
<td>E7</td>
<td>832</td>
<td>A $\leftrightarrow$ G</td>
<td>Glutamic acid</td>
</tr>
</tbody>
</table>

of the L2-ORF, the entire L1-ORF, the non-coding region and the E6-ORF (nucleotide positions 5144–658) of HPV-18. Screening of $10^5$ recombinant phages led to the identification of a single positive recombinant clone that carried an insert of about 9.5 kb. This insert was subcloned into the plasmid vector Bluescribe (Stratagene) for further analysis. DNA sequence analysis was performed by the dideoxy method (Sanger et al., 1977) by using the oligonucleotide primers M13 reverse and M13 universal, which flank the multiple cloning site of the vector. The first 50–200 nucleotides at each end of the 9.5 kb insert were determined, and the sequence data were analysed by use of the HUSAR (Heidelberg Unix Sequence Analysis Resources) program package. DNA sequence comparison revealed no identities to any HPV DNA. Instead, identities to human Alu-repeat sequences were found (Batzer et al., 1996). In order to determine the identity of the HPV sequences that form part of the 9.5 kb fragment, it was decided to amplify a region within the putative viral E6-ORF. This was achieved by using a set of consensus primers specific for the E6-ORF of high-risk HPV types (Lungu et al., 1995). A fragment of 200 bp was amplified and cloned into a plasmid vector (TA-Cloning Kit, Invitrogen). The complete 200 bp fragment was sequenced and was shown to share 99.5% identity with HPV-45 DNA (Naghashfar et al., 1987). A single nucleotide exchange,
A consequence of integration (Schwarz et al., 1987) revealed that HPV-45 is most closely related to HPV-18 (73.9%), followed by HPV-39 (59.2%) (Bernard et al., 1994; Delius & Hofmann, 1994). The previous assumption that MS751 cells harbour HPV-18 DNA (Pater & Pater, 1985; Yee et al., 1989) can be explained by cross-hybridization between both HPV types because of the close sequence relationship (Naghashfar et al., 1987). It should be noted that in the early eighties, when cervical carcinoma cell lines were screened for the presence of HPV-specific sequences, HPV-16 and HPV-18 were the only oncogenic HPV types available as molecularly cloned probes (Boshart et al., 1984; Tümmler et al., 1983).

HPV-45 is a cancer-associated type first cloned from a recurrent cervical lesion displaying mild to moderate dysplasia with koilocytosis (Naghashfar et al., 1987). Its prevalence in genital tract infections is rather low (Naghashfar et al., 1987; Williamson et al., 1994). However, there seems to be significant geographical variation. A clustering of HPV-45 was noted for Western Africa (Boshart et al., 1995). Sequence comparison of HPV-45 from the cervical carcinoma cell line MS751 with the originally cloned isolate (Naghashfar et al., 1987) revealed some interesting differences at the amino acid level of the E7 gene. Three hydrophilic amino acids at the amino-terminal end of the protein which are characteristic for the original HPV-45 isolate are replaced by hydrophobic amino acids (Table 1). It is likely that these alterations have an effect on the functional properties of the protein. It will now have to be investigated whether these differences relate in any way to the transforming potential of HPV-45. Moreover, the existence of two HPV-45 strains with different biological potential should be considered.

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References


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