Identification of Papillomaviral DNA Sequences in Hairless Mouse Tumours Induced by Ultraviolet Irradiation

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SUMMARY

Papillomas, carcinomas in situ and squamous cell carcinomas were induced using ultraviolet irradiation in the hairless mouse strain Mus musculus HRA/Skh. DNA extracted from biopsies was examined using Mastomys natalensis papillomaviral DNA as a hybridization probe at reduced stringency. Sequences homologous to the probe were detected in 16 of 24 papillomas, five of five carcinomas in situ and six of 38 squamous cell carcinomas. A number of tumour DNAs (16/33) also hybridized with mixed DNAs of human papillomavirus types 11, 13, 16 and 18 at reduced stringency. This suggests a role for the hairless mouse as a laboratory model for the study of the involvement of papillomaviruses in malignant transformations.

Papillomaviruses (PVs) cause benign proliferations of the skin and mucosa and have been associated with squamous cell carcinomas (SCCs) in some vertebrates (Lancaster & Olson, 1982; Gissmann, 1984; Pfister, 1984). A major obstacle to the study of papillomaviruses, which in general appear to be highly species-specific, has been the lack of a tissue culture system or an appropriate laboratory animal in which to propagate and study the virus. At present, papillomaviruses have been isolated and cloned from only two rodent species, the North African rat Mastomys natalensis (Mn) (Amtmann et al., 1984; Müller & Gissmann, 1978) and the European harvest mouse Micromys minutus (Mm) (Sundberg et al., 1987; O'Banion et al., 1988). Although rodents, these two species have limited use as laboratory animal models for the study of papillomaviruses. The animals are not available as inbred strains and, in the case of M. minutus, the experimental induction of tumours has not been established. The induction of papillomas by ultraviolet irradiation followed by malignant progression to SCCs has been reported in the hairless mouse strain Mus musculus HRA/Skh (Gallagher et al., 1984; Reeve et al., 1985). We report here, on the basis of hairless mouse DNA analyses using MnPV as a hybridization probe, the first indication of association of the skin tumours in the hairless mouse with a papillomavirus. The presence of MnPV-like DNA sequences in u.v.-induced skin tumours of the hairless mouse suggests these mice could be developed as a laboratory model to study the role of papillomaviruses in malignant transformation.

Tumours on the skin of hairless mice were induced by u.v. as previously described (Reeve et al., 1985) and typed grossly according to the classification of human skin tumours (Gallagher et al., 1984). The tumours commonly appeared first as papillomas 100 days after a 10 week regime of a daily minimally erythematous dose of u.v. Weekly mapping and histological confirmation determined that many of the papillomas progressed to malignancy through carcinomas in situ (CIS) and keratoacanthoma (KA)-like forms for which SCC represented the end stage.
Fig. 1. Detection of MnPV-like DNA sequences in individual tumours obtained from five different hairless mice after hybridization with MnPV DNA at $T_m = 30 \degree C$. Lane 1, 10 pg MnPV insert DNA removed from pBR322 at its single HindIII site; lanes 2 to 6, EcoRI-digested DNA (10 µg) of a spontaneous SCC (lane 2), a DMBA-induced papilloma (lane 3), a u.v.-induced papilloma (lane 4), a u.v.-induced SCC (lane 5) and a u.v.-induced CIS (lane 6). Sequences were also detected at $T_m = 20 \degree C$ in the tumours represented in lanes 2, 3 and 6.

Fig. 2. Detection of HPV-like DNA sequences in two tumours removed from the same u.v.-irradiated mouse. Hybridization was performed at $T_m = 33 \degree C$ using HPV types 11, 16 and 18 as a combined hybridization probe. Lane 1, 10 pg HPV-16 insert removed from pBR322 at its single BamHI site; lanes 2 and 3, EcoRI-digested DNA (10 µg) of a u.v.-induced SCC (lane 2), and a u.v.-induced papilloma (lane 3).

(Gallagher et al., 1984). However, CIS, KAs and SCCs also arose directly from u.v.-damaged skin. U.v.-treated and untreated normal skin samples were obtained at the same time and used as controls in hybridizations.

Biopsies (81) were removed from 60 animals, frozen in liquid nitrogen and freeze-dried. DNA was isolated by the method of Krieg et al. (1983) either from individual tumours or, in some cases, from a pool of several samples of the same tumour type when samples were too small (< 10 mg) to process individually. DNA (10 µg) was digested with restriction enzyme EcoRI (2 units/µg; 37 °C overnight) and transferred to GeneScreen Plus membrane by alkaline transfer (Chomczynski & Qasba, 1984). The membranes were hybridized using MnPV DNA, which had been excised from its pBR322 plasmid and labelled with [$\alpha^{32}$P]TTP to a specific activity of $10^8$ to $2 \times 10^8$ d.p.m./µg (Kulski et al., 1987) (Fig. 1). The results obtained from the hybridization analyses of 14 normal skins and 67 skin tumours from 60 hairless mice are presented in Table 1. Initially, MnPV-like DNA sequences were detected in two of 31 tumours, a u.v.-induced CIS and a spontaneous SCC, at high stringency ($T_m = 30 \degree C$). When the stringency of hybridization was decreased to $T_m = 20 \degree C$ (Pfister, 1984), the overall number of positive samples increased to 27 of 67 tumours (40%). MnPV-like sequences were detected in 16 of the 24 papilloma samples, all five CISs and six of the 38 SCCs. Sequences homologous to MnPV were not detected in any of the samples of normal skin obtained from 14 hairless mice nor in the skins of four BALB/c mouse samples (data not shown).

Single biopsies were removed from 33 mice of which none of seven normals, five of 11 papillomas, all of five CISs and three of 10 SCCs were positive with the MnPV probe (Fig. 1, lanes 2, 4, 5 and 6). Another 48 biopsies were removed from 27 mice of which MnPV-like sequences were detected in none of seven normals, 11 of 13 papillomas and three of 28 SCCs.
Table 1. Hybridization analysis at $T_m - 30^\circ C$ of normal and neoplastic hairless mouse skin using MnPV DNA as a hybridization probe

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Number of positives/number of samples tested</th>
<th>Number of mice*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-irradiated</td>
<td>U.v.-irradiated</td>
</tr>
<tr>
<td>Normal</td>
<td>0/9</td>
<td>0/5</td>
</tr>
<tr>
<td>Papilloma</td>
<td>0/1</td>
<td>16/23</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td>5/5</td>
<td>5/37</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>1/1</td>
<td>5/37</td>
</tr>
</tbody>
</table>

* A total of 81 skin samples were obtained from 60 mice; normal and neoplastic samples from multiple locations on the mouse were obtained from four of the 60.

An analysis of multiple tumours from four mice showed that an individual mouse could bear both positive and negative tumours. In the case of one mouse, both papillomas contained MnPV-like sequences whilst three normal skins were negative (data not shown). No MnPV sequences were detected in a papilloma or in one or two SCCs which were each removed from another two mice. In the case of a fourth mouse, MnPV-like sequences were detected in a papilloma and one of two SCCs (data not shown).

A total of 15 papillomas were also obtained from 12 hairless mice treated with 9,10-dimethyl-1,2-benzanthracene (DMBA) (eight samples) or 3,4-benzpyrene (seven samples), with or without croton oil promotion. Of these 15 papillomas, four contained MnPV-like sequences detectable at high stringency (data not shown) and another three were positive when the stringency was reduced to $T_m - 30^\circ C$ (Fig. 1, lane 3, DMBA-induced papilloma).

The detection of MnPV-like DNA in hairless mouse tumours at high stringency indicates that in these mice a PV DNA is present with nucleotide sequences which are either closely related to MnPV or minimally related but at a highly amplified copy number in certain tumours. On the basis of limited restriction enzyme analyses, the hairless mouse DNA sequences and MnPV appear to be different. This difference is reflected in the single EcoRI site of the MnPV which produces an 8 kb fragment (Amtmann & Wayss, 1986) whereas variable sizes were obtained with EcoRI for the DNA sequences detected in five biopsies which were collected individually from five hairless mice (Fig. 1). Fragments of 30, 15 and 8 kb were obtained in a spontaneous SCC and a DMBA-induced papilloma (lanes 2 and 3), but only the 15 kb fragment was present in a u.v.-induced papilloma and SCC (lanes 4 and 5). In a u.v.-induced CIS (lane 6), fragments of 15 kb and 4 kb were present. The presence of the larger fragments of DNA (15 and 30 kb), after an EcoRI digestion, suggests that the DNA does not exist simply as a monomeric form but may also be present in a dimeric or trimeric form whether in an integrated or episomal state.

The incidence of detection of the MnPV-like DNA sequences was lower for SCCs (16%; n = 38) than for papillomas (67%; n = 24) or CISs (100%; n = 5). In addition, no endogenous sequences were detected in the 14 normal samples which is in contrast to the M. natalensis (Amtmann et al., 1984) and M. minutus (Sundberg et al., 1987) PV models. This lower incidence in part may be explained by the presence of a lower copy number of PV sequences in the normal tissues and in SCCs than in the papillomas and CISs; these sequences might be below the limit of sensitivity of our assay [10 pg MnPV DNA; 1000 pg human PV (HPV-16) DNA] using MnPV as a hybridization probe at reduced stringency ($T_m - 30^\circ C$). Alternatively, several types of hairless mouse PV may exist with one PV type predominating in a particular lesion as has been found in human genital lesions. Human PV types 6 and 11 predominate in condyloma acuminatum whereas HPV types 16 and 18 predominate in flat condylomas and SCCs of the uterine cervix (Gissmann et al., 1983; Kulski et al., 1987). Hybridization using a mixed HPV (types 11/13/16/18) DNA probe at reduced stringency ($T_m - 33^\circ C$) revealed the presence of HPV-like DNA sequences in three of 17 papillomas, five of five CIS and eight of 11 SCCs. Of these 16 HPV DNA-positive biopsies, 10 had also hybridized with MnPV DNA. However, one papilloma and five SCCs had not previously hybridized with MnPV DNA. DNA fragments of 15 kb and 8 kb which hybridized with a mixed HPV (types 11/16/18) probe were detected in a
Short communication

u.v.-induced papilloma and SCC, obtained from the same animal (Fig. 2, lanes 2 and 3). These sequences had not cross-hybridized previously with the MnPV DNA probe suggesting the presence of a different type of hairless mouse PV infecting this particular mouse.

So far viral particles have not been detected in any u.v.-induced tumours of the hairless mouse when examined by immunohistochemistry with antisera raised against bovine PV-1 common capsid antigen or electron microscopy. However, DNA extracts from such tumours, when applied topically, have shown enhancement of u.v. carcinogenesis in the skin of the hairless mouse (V. E. Reeve, G. E. Greenoak, P. J. Canfield, C. Boehm-Wilcox, P. A. Tilbrook, J. K. Kulski & C. H. Gallagher, unpublished data) indicating the possible transfer of infective transforming sequences.

The cross-hybridization between MnPV probe and DNA sequences in the hairless mouse tumours using conditions of high and moderate stringency indicates the presence of PV-like sequences in tumours of the hairless mouse. The cloning and characterization of these mouse sequences is in progress for their eventual use as specific probes to determine distribution and physical state of the sequences in the hairless mouse tumours.

Ultraviolet light is an accepted causative agent in the malignant transformation of the skin in man and animals (Forbes et al., 1979). Therefore, if the hairless mouse contains PV-like DNA in u.v.-induced benign tumours which progress towards malignancy, as has been inferred in this report, then the inbred hairless mouse M. musculus HRA/Skh could be a useful laboratory animal model with which to study the association between u.v., PVs and the immune response elicited by the PVs, within the malignant transformation of the skin cancers.

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Short communication


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