Papillomavirus DNA Associated with Pulmonary Fibromatosis in European Elks

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Multiple beadlike fibromas have been observed in the lungs of European elks bearing cutaneous fibromas and fibropapillomas. DNA extracted from the lung fibromas was found to contain multiple copies of unintegrated European elk papillomavirus DNA, indicating an association between pulmonary fibromatosis and papillomavirus. No virus particles were observed in the tumor tissue by electron microscopy. Histological examination revealed that the lung fibromas had a morphology similar to that of cutaneous fibromas.

Cutaneous warts are frequently seen in the Swedish population of European elks (Alces a. alces) (1). We have previously shown that they are caused by a newly discovered member of the papillomavirus family, the European elk papillomavirus (EEPV) (5). The cutaneous warts in elks were characterized morphologically as fibropapillomas or fibromas. Fibropapillomas are fibroblastic tumors covered by a roughened, pigmented epithelial layer, whereas fibromas consist almost exclusively of fibromatous tissue with or without a thin epithelial layer. Papillomavirus particles are only found in the highly keratinized epithelial layer, whereas no virus particles have been isolated from fibromas. Papillomavirus DNA is, however, consistently found also in these tumors by Southern blot hybridization (unpublished observations).

We have in rare cases observed that European elks, bearing cutaneous tumors also have beadlike fibromatous tumors in the lungs. Since 1960 only four animals with lung tumors in connection with cutaneous warts have been observed in necropsy material sent to our laboratory. Lung tumors with a similar appearance have also been reported in the white-tailed deer (3). The etiology of pulmonary fibromas has, however, not been clarified.

In the present communication we report the presence of beadlike pulmonary fibromas in an elk which carried multiple cutaneous fibromas and fibropapillomas. Southern blot hybridization revealed the presence of multiple copies of unintegrated EEPV DNA in the lung tumors, suggesting that EEPV in rare cases causes lung fibromatosis. Papillomaviruses have previously been reported to be associated with tumors in many different organs (for a review, see reference 6). The present report is, to the best of our knowledge, the first demonstration of an association between a papillomavirus and a nonmetastatic lung tumor.

An approximately 4-year-old European elk, apparently in good health although bearing a few cutaneous fibromas and fibropapillomas, was shot in the northern part of Sweden. When the elk was eviscerated, multiple beadlike tumors were observed in the lungs, whereas other visceral organs were free of macroscopically detectable neoplasms. Parts of the lungs were sent from the field to the National Veterinary Laboratory for further analysis. Small portions of the lung tumors were fixed in 10% formaldehyde for histological examination, and the rest of the material was used as a potential source for isolation of virus particles and viral DNA. The macroscopic examination of the lungs revealed the presence of multiple white nodules (Fig. 1) that varied in diameter from less than 1 mm to approximately 10 mm. Several small nodules could also be palpated deeper in the lung and could be exposed by dissection. The nodules had a characteristic rubberlike texture and were easily separable from the surrounding lung tissue by manual pressure. No similar nodules were found in other organs as judged by visual inspection and palpation.

Histologically, the lung nodules were typical fibromas consisting of loose to dense bundles of connective tissue, sparsely vasculated with few nuclei. The mitotic activity was found to be low. The nodules were located in the alveolar part of the lung, compressing the surrounding tissue. In many cases the tumor tissue seemed to be attached to the lung tissue via a narrow stalk. No signs of infiltrative growth were observed, suggesting that the tumors were benign (Fig. 2).

The fibromas were also subjected to an electron microscopic examination; nodules were manually separated from the lung tissue, and a 10% suspension in phosphate-buffered saline was prepared by mincing and homogenizing in an Ultraturrax mixer. After repeated low-speed centrifugations the supernatant was pelleted by high-speed centrifugation. The pellet was then suspended in a small volume of buffer containing 0.01 M Tris hydrochloride (pH 7.5) and 0.001 M EDTA (TE buffer). One drop of the suspension was placed on a Formvar-copper grid and negatively stained with 1.5% ammonium molybdate. Electron microscopic examination of several grids failed to reveal virus particles with papillomavirus morphology, even after an extensive search.

It was anticipated that an association might exist between the lung fibromas and EEPV, a virus known to induce benign cutaneous tumors in European elks. (5). Therefore DNA from the lung tumors was examined for the presence of EEPV sequences by hybridization. Approximately 10 g of tumor tissue or adjacent normal tissue was minced separately to a homogeneous mass, and DNA was extracted as described by Steffen and Weinberg (10). The purified DNA
was cleaved with restriction endonuclease BglII before separation in a 1% agarose gel. The separated fragments were blotted onto a nitrocellulose membrane by the method of Southern (9), and fragments containing EEPV DNA were detected by hybridization with a probe consisting of the EEPV genome cloned in the BamHI site of the pBR322 vector (11). The probe hybridized strongly to three fragments in the tumor DNA of 4.0, 2.1, and 1.7 kilobases. These fragments were identical in size to fragments generated from the EEPV genome with the corresponding restriction enzyme (11) (Fig. 3). Hybridization was detected with a similar intensity after washing under stringent hybridization conditions, i.e., at 65°C in 0.1 SSC (1 X SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The results thus show that EEPV DNA is present in the lung tumors, and from the restriction enzyme cleavage pattern it can be predicted that the viral DNA is present in an uniterated form. DNA from the normal surrounding tissue, in contrast, revealed no hybridization.

The number of viral DNA copies that were present per tumor cell was estimated by performing reconstruction experiments, i.e., hybridization to a mixture on DNA from normal lung tissue and calibrated amounts of cloned EEPV DNA. We found that 100 to 500 viral genomes were present per cell on an average.

Members of the papillomavirus family are oncogenic viruses with a wide host range. The cottontail rabbit papillomavirus for example, causes cutaneous papillomas in domestic as well as cottontail rabbits. After a period of rapid growth, the tumors can either regress or undergo transformation to carcinomas in cottontail rabbits. They metastasize to the lungs and other organs, eventually causing the death of the animal (2, 8, 13). It has moreover been possible to establish transplantable carcinoma cell lines from rabbits infected with cottontail rabbit papillomavirus (8). When transplanted into the thigh muscles of rabbits, these cell lines (VX2 and VX7) were frequently found to form metastatic tumors in the lungs. The histology of the lung tumors was similar to that of primary squamous lung carcinomas, observed after infection with cottontail rabbit papillomavirus. Lung tumors have also been observed after intravenous inoculation of the VX2 carcinoma cell line (4). Lung metastases have furthermore been observed in Syrian hamsters after subcutaneous inoculation with bovine papillomavirus (7). Koller and Olson (3) reported the occurrence of lung fibroblastomas in a white-tailed deer with cutaneous fibromas. The cutaneous and lung tumors had a similar histology, and the authors suggested that metastasis to the lungs had occurred and that the virally induced cutaneous tumors are potentially malignant. However, no conclusive evidence was provided for a viral etiology of the lung tumors, since no DNA probes were available at that time for hybridization analysis. The pulmonary fibroblastomas in the white-tailed deer and the pulmonary fibromas in the European elk are, to the best of our knowledge, the only examples of benign papillomavirus-associated tumors of internal organs. Metastatic tumors have been observed in cottontail rabbits as well as in hamsters, and it has also been reported recently that human papillomavirus type 16 DNA sometimes is present in human lung carcinomas (12).

The necropsy findings and the histological pictures are similar in both the white-tailed deer and the European elk. The elk pulmonary fibromas show, however, no signs of infiltrative growth and are therefore unlikely to be metastases originating from the cutaneous tumors.

The presence of EEPV DNA in the tumor tissue and the
FIG. 2. (A) Section through lung tissue with a large fibroma, consisting of bundles on connective tissue. The tumor is not attached to the surrounding lung tissue. Van Gieson. ×250. (B) Section through lung tissue with a small fibroma. The upper part of the tumor is attached to the lung tissue, but the growth does not appear to be infiltrative.
Additional factors seem thus to be required for EEPV to induce lung tumors.

Experimental transmission of the disease should provide additional information on the sequential stages and etiology of the disease. It might also be rewarding to search for similar tumors in other animal species.

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