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HETEROTRANSPLANTATION OF ARGYROPHIL SMALL CELL CARCINOMA OF THE UTERINE CERVIX INTEGRATING HPV16 DNA INTO NUDE MICE

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Argyrophil small cell carcinoma of the uterine cervix (ASCC) containing human papillomavirus type 16 (HPV16) DNA has been successfully transplanted in nude mice for the first time, and we have designated the resultant cell line as YIK-1. Histology of YIK-1 was similar to that of the original tumor. The original and YIK-1 tumor cells contained argyrophil granules and neurosecretory granules in the cytoplasm, and were immunohistochemically stained positive for neuron-specific enolase, serotonin and chromogranin. Both tumors contained HPV16 DNA in a multiple-copy integrated form. Thus, YIK-1 maintains the characteristics of the original ASCC, and may therefore be useful as an animal system for experimental studies of ASCC.

Key words: Heterotransplantation — Argyrophil small cell carcinoma — Human papillomavirus type 16

Argyrophil small cell carcinoma of the uterine cervix (ASCC), which was considered to be derived from the APUD (amine precursor uptake and decarboxylation) cell system,[1] was first described by Albores-Saavedra and co-workers in 1972.[2] Although ASCC is a rare cervical tumor, the poor prognosis of ASCC has made recognition of this type of cervical carcinoma of great clinical significance.[1,3,4] An *in vivo* experimental system has, therefore, been desired for the purpose of characterizing this malignant tumor and testing chemotherapeutic regimens.

A possible role of human papillomavirus (HPV) infections in the genesis of cervical cancer, especially squamous cell carcinoma, has been extensively discussed during recent years.[5,6] However, little attention has been paid to other histological types of uterine cervix carcinomas.

This is the first time that a rare cervical tumor, ASCC, containing HPV16 genomes in a multiple-copy integrated form has been successfully transplanted into nude mice.

The tumor tissue was obtained by surgical operation from a 31-year-old woman with ASCC brain metastatic lesions. She died of multiple organ metastases 2 years after hysterectomy and lymphadenectomy for clinical stage Ib of ASCC. The tumor was cut into small pieces in Eagle's minimum essential medium and transplanted subcutaneously using a trocar needle onto the backs of 4–5-week-old BALB/c female nude (*nu/nu*) mice (Clea Japan Inc., Japan). Serial transplantation of the tumor was carried out similarly.

Fresh tumors taken from the brain metastatic lesion of the patient with ASCC and the tumor-bearing mice were subjected to light and electron microscopic observations, and Southern blot hybridization analysis. For light microscopic studies, sections of the tumor tissues were fixed in 10% formalin, stained with hematoxylin and eosin, processed by the Grimelius method or stained immunohistochemically by the avidin-biotin-peroxidase complex method using antibodies to neuron-specific enolase (NSE), gastrin, carcinoembryonic antigen, adrenocorticotropic hormone, chromogranin, somatostatin (Lipshaw Co., USA), and serotonin (Sera-lab Ltd., UK). For electron microscopic studies, the specimens were fixed in a 2.5% glutaraldehyde solution followed by 1% osmium tetrox-

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ide, embedded in Epon, and stained with uranyl acetate and lead citrate. Ultrathin sections were observed with a Hitachi HU-12A electron microscope. For Southern blot analysis, genomic DNA was extracted using the method described by Maniatis *et al.*[7] The genomic DNAs were digested with *Bam*HI, *Bgl*II or *Pst*I supplied by Toyobo Co., Ltd., Japan. The digested DNAs were electrophoresed in 0.6–1.5% agarose gels and transferred to nylon membranes (Zeta Probe; Bio-Rad, USA).[8] The membranes were prehybridized in 1.5×SSPE (1×SSPE: 0.15*M* NaCl, 0.01*M* NaH_2PO_4, 0.001*M* EDTA), 1% sodium dodecyl sulfate (SDS), 0.5% nonfat powdered milk and 500 μg/ml denatured salmon sperm DNA for 4 hr at 68°. Hybridization was performed at 68° overnight, in the above solution containing 10% dextran sulfate and the probe, which consisted of HPV 6, 11, 16 or 18 DNA excised from the plasmid clones provided by Dr. H. zur Hauzen with *Bam*HI or *Eco*RI, and were labeled with [^{32}P]-dCTP to the specific activity of 5–8×10^8 cpm/μg DNA using the procedure of Feinberg and Vogelstein.[9] The membranes were washed in 2×SSC (1×SSC: 0.15*M* NaCl, 0.015*M* sodium citrate)/0.1% SDS, 0.5×SSC/0.1% SDS and 0.1×SSC/0.1% SDS at room temperature for 15 min each, followed by a wash in 0.1×SSC/1% SDS at 50° for 30 min. Autoradiographs were prepared on Fuji X-ray films.

When ASCC was transplanted into nude mice, the transplants grew as solid tumors at the inoculated site. These transplanted tumors were designated as YIK-1. YIK-1 has been passed nine times in nude mice so far, and the tumor take rate was more than 90%. Histological examination of the original ASCC revealed sheets of small, round, poorly

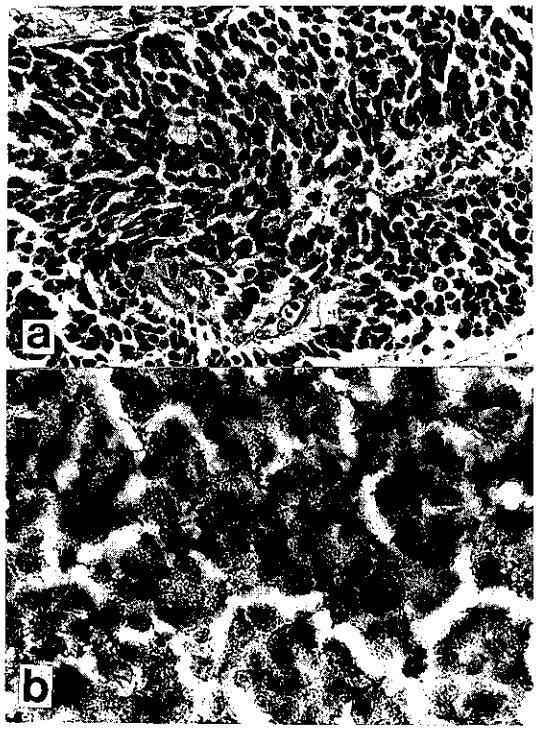

Fig. 1. (a) Hematoxylin and eosin stain (×132) and (b) Grimelius stain (×660) of the original ASCC.

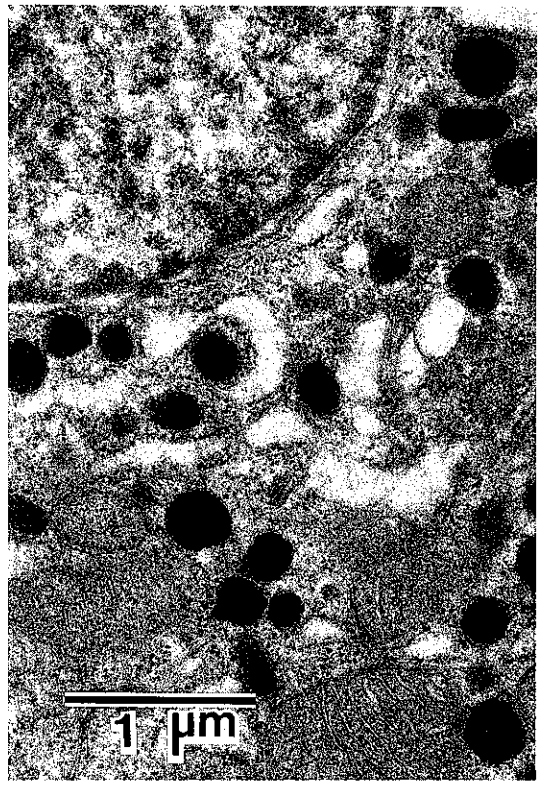

Fig. 2. Electron micrograph showing dense-core membrane-bound granules in the cytoplasm of YIK-1 tumor cells from the seventh *in vivo* passage (magnification ×15,000).

differentiated cells with large ovoid hyperchromatic nuclei and scanty cytoplasm (Fig. 1a). The histological appearance of YIK-1 at the fourth passage was similar to that of the original tumor. Some cells from both tumors contained diffusely distributed argyrophil granules in the cytoplasm (Fig. 1b). Immunohistochemical studies of both the original and YIK-1 tumor cells demonstrated the presence of NSE, chromogranin and serotonin. Electron microscopic examination demonstrated several small electron-dense membrane-bound neurosecretory-type granules in some cells of both tumors (Fig. 2). Thus, YIK-1 retains the characteristics of the original ASCC well.

DNAs from the ASCC brain metastatic tumor and YIK-1 at the fourth passage were analyzed for the presence of HPV DNA. Both tumors contained more than 1000 copies of HPV16 DNA per diploid cell, and this DNA co-migrated with cellular DNA, suggesting that the HPV16 sequences were integrated into the host genome (Fig. 3, lanes e, f and g). HPV 6, 11 and 18 DNAs were not detected (data not shown). The gross structure and organization of HPV16 DNA were determined in the original and YIK-1 tumors by digesting cellular genomic DNA either with an enzyme which should not cleave HPV16 DNA (*Bgl*II) or with enzymes which cleave the viral genome (*Bam*HI, once or *Pst*I, six times) and hybridizing with radiolabeled HPV16 DNA. On digestion with *Bgl*II, two hybridizing fragments (one strong band and one weak band) greater than the HPV16 unit length (7.9 kilobase pairs (kb)) were present, suggesting that the viral sequences were integrated into at least two sites of the host cell genome (Fig. 3, lanes c and d). Digestion of both genomic DNAs with *Bam*HI revealed three hybridizing fragments, none of which corresponded to the band with the mobility of linear unit length of HPV16 DNA, confirming that the virus was integrated into the host cell genome (Fig. 3, lanes a and b). Hybridization of the DNA probe with *Pst*I-cleaved tumor cell DNA revealed five of the six anticipated fragments (1.78, 1.55, 1.06, 0.48 and 0.22 kb)[6] (Fig. 4). The absence of the 2.82 kb *Pst*I fragment and the presence of two new fragments suggested that integration had occurred within this region of the viral genome. There

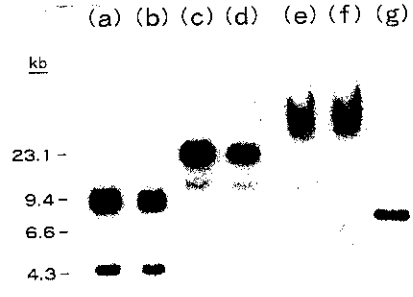

Fig. 3. Blot hybridization of ^{32}P-labeled HPV16 DNA to DNAs from the ASCC brain metastatic lesion (lanes a, c and e) and from the fourth YIK-1 *in vivo* passage (lanes b, d and f) after cleavage with *Bam*HI (lanes a and b), or *Bgl*II (lanes c and d), or without prior treatment with any restriction enzymes (lanes e and f). The digested and undigested DNAs (1 μg) were electrophoresed in 0.6% agarose gels. Lane g contained 2.6 ng (approximately 1000 copies per cell) of cloned HPV16 DNA freed from the cloning vector by cleavage with *Bam*HI.

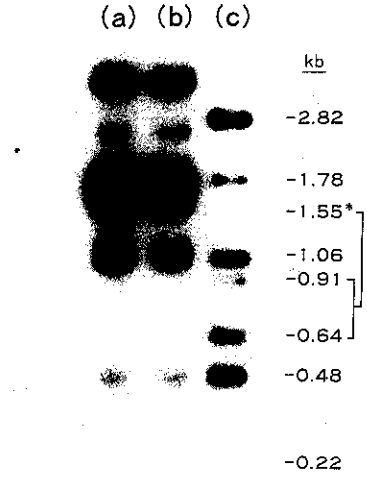

Fig. 4. Blot hybridization of ^{32}P-labeled HPV16 DNA to *Pst*I-digested DNAs from (a) ASCC brain metastatic lesion and (b) the fourth YIK-1 *in vivo* passage. Cellular DNAs (2.5 μg) were electrophoresed in 1.5% agarose gels after digestion with *Pst*I. (c) Prototype HPV16 fragments obtained by *Pst*I-*Bam*HI digestion of a HPV16 plasmid were used as electrophoretic markers. The prototype 1.55-kb *Pst*I fragment (asterisked) was cleaved at the cloning restriction site (*Bam*HI) into 0.91- and 0.64-kb fragments.

was no difference in the integration profiles of HPV16 DNA between the original ASCC and YIK-1.

We established a new nude mouse/human tumor xenograft system, YIK-1, which was histologically (Figs. 1 and 2) and virologically (Figs. 3 and 4) found to maintain the characteristics of the original ASCC. There has been no previous report of ASCC tumor tissue being successfully transplanted into nude mice. Despite the declining incidence and mortality of cervical cancer, as a result of early detection programs in many developed countries, the prognosis of ASCC is still poor.[3] Because of its rarity,[1,4] most of the reports on ASCC have been concerned with single cases or small series of cases. This system may therefore be useful not only for characterizing ASCC, but also in searching for an effective clinical treatment of ASCC.

It has been suggested that the presence and integration of HPV16 is a risk factor for the development of cervical cancer,[5] and that the E6 and/or E7 open reading frames (ORFs) may be potential oncogenes essential in some stages of HPV-induced cellular transformation and may also be needed for the maintenance of the transformed phenotype.[10] In this study, not only the original ASCC, but also YIK-1, even after several passages in nude mice maintained HPV16 DNA in a multiple-copy integrated form, and both tumors retained the 1.78-kb *Pst*I fragment of HPV16 DNA, which contains the E6 and E7 ORFs, and the long control region. These results suggest that HPV16 DNA may play a role in the genesis of this malignant tumor and/or in the maintenance of the transformed phenotype of YIK-1 directly or by cis- or trans-activation of some cellular oncogenes,[11] and that YIK-1 could be useful as an animal system for investigating the relationship between HPV16 and ASCC.

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