TABLE 1. Assessment of Degree of Periodicity in Arrangement of Leukemic Cells on the Basis of Statistical Analysis of Distances between Cells

Degree of periodicity	Arithmetic mean and standard deviation, μ	Probability of significant difference between samples relative to difference of standard deviation
High	58,8±4,2	0,999
Av. (intermediae)	54,5±9,4	0,7
Chaos	49,1±12,7	—

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TRANSPLANTABLE XENOGRAFTS OF HUMAN CERVICAL CARCINOMA. CHARACTERIZATION OF HUMAN PAPILLOMAVIRUS GENOMES

S. A. Galetskii, V. N. Kopyl'tsov, K. I. Zhordaniya, Yu. N. Solov'ev, and E. S. Revazova

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The link between papillomaviruses and human tumor pathology is one of the most important topics at the present time in oncovirology. The genetic information of human papillomaviruses (HPV) has been found by molecular-biological methods and cloned from cells of various human neoplasms, both benign and malignant. One of the latter is carcinoma of the uterine cervix (CUC).

More than 50 types of HPV genomes have now been cloned [12]. In human CUC cells, HPV of type 16 (HPV-16) has most frequently been found, but types HP-18, 31, 33, 35, and 39 also have been described [4, 5, 12]. According to evidence obtained [5, 12], DNA of HPV can be found in clinical specimens of human CUC with a frequency of between 34 and 90%. The oncogenic potential of DNA of HPV, and in particular, of HPV-16, is proved by the discovery of the sequences of this virus after transfection of DNA of clinical material of CUC into NIH/3T3 cells [14], and by the ability of the cloned HPV-16 genome to transform human keratinocytes and fibroblasts [8].

The oncogenic potential of HPV DNA and the frequent association of HPV with CUC suggest that papillomaviruses play a role in the oncogenesis of the epithelium of the uterine cervix. A penetrating study of this problem can be undertaken most effectively on model systems. The best known are eight cell lines of human CUC, contained in the American Type Culture

All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

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TABLE 1. Clinical Data for Patients from Whom Xenografts Were Obtained

Model	Clinical diagnosis	Stage of disease	Histology	Age	Treat before biopsy	Outcome
CUC-5	$T_{2AB}N_1M_0$	IIAB	Squamous-cell carcinoma	37	—	Died from primary disease
CUC-9	$T_{1B}N_0M_0$	IB	The same	38	—	Without progression of primary disease
CUC-24	$T_2ABN_1M_0$	IIAB	» »	24	—	Died from primary disease
CUC-25	$T_{1Б}N_0M_0$	IB	» »	52	—	Unknown

Collection [14], five cell lines of CUC obtained in Japan [9, 13], and four new lines of human CUC, obtained in Great Britain, recently described [7, 11].

Models of human CUC, transplantable in vivo and obtained both from a cell line and from clinical material, have been created and described previously in the Laboratory of Experimental Models, All-Union Oncologic Scientific Center [1, 3]. We now describe three new models of human CUC, transplantable into nude mice, obtained exclusively from clinical material, and we give data on the presence and structure of the genetic information of the HPV in these tumors, and also in the CUC-1 and CUC-5 models described previously.

EXPERIMENTAL METHOD

Nude mice based on the BALB/c line, reared by ourselves, were used. Transplantations were carried out by the method described previously [1, 3]. Histological preparations were obtained by the usual method [1] and stained with hematoxylin and eosin.

DNA was isolated from the tumors by the method in [2], using treatment with pronase and sodium dodecylsulfate, followed by deproteinization with phenol and chloroform. Fragmentation of the DNA was carried out by restriction endonucleases from the "Ferment" Research and Production Combine (Vilnius) under conditions recommended by a producer. Fractionation and transfer of the DNA to nitrocellulose ("Schleicher und Schuel," West Germany) were carried out as in [10].

The filters were hybridized in buffer of the following composition: 5×SSC, 2× Denhardt's solution, 50% formamide, 250 μg/ml of yeast *t*RNA, 0.05 M Na-phosphate buffer, pH 6.5, 0.1% Na dodecylsulfate, (2-3) · 10^6 cpm/ml of ^{32}P-labeled plasmid DNA, at 42°C for 24-48 h.

DNA from HPV-16 and HPV-18, supplied by Df zur Hausen (West Germany), were obtained from Professor F. L. Kiselev. Plasmid DNA was labeled with ^{32}P-dNTP in the nick translation reaction (the radionuclides were obtained from the "Radiopreparat" enterprise, Institute of Nuclear Physics, Academy of Sciences of the Uzbek SSR). The virus DNA was first excised from the plasmid, separated in gel, and removed, using a DEAE-membrane and the method described by the firm "Schleicher und Schuel" (West Germany). Specific activity of the sample was 2 · 10^8 cpm/μg DNA.

The blots were eluted successively with buffers containing 2×SSC, 0.1% SDS; 1×SSC, 0.1% SDS; 0.1×SSC, 0.1% SDS, at temperatures of 42 and 67°C.

Autoradiography was carried out on film from the firm ORWO (East Germany) at 70°C, with an exposure of several days.

EXPERIMENTAL RESULTS

Biology of the Models. As the data in Table 1 show, xenografts were obtained from patients in whom the disease varied in severity and in the degree of its manifestations. In two cases the patients were in stage IIAB, which was manifested as regional metastases, and the patients soon died. One patient was young (aged 24 years), and the disease in such cases is known to follow a particularly aggressive course. However, aggressiveness of the tumors in the patients did not correlate with the time of onset, the rate of growth, and the probability of survival of primary xenografts in mice (Table 2). Growth of the tumor in nude mice is evidently not determined by the stage of the process at which malignant tissue was obtained from the patient.

The models have now gone through 8-38 transplantations (Table 2). The mean time between transplantations is 14-18 days. During this time the mass of the tumor reaches 1-1.5 g. The period of survival of mice with a tumor is 21-35 days. Metastasization was not observed.

TABLE 2. Biological Characteristics of Models

Name of model	Primary success rate of transplantation	Time of first transplantation*	Number of transplantations
CUC-1 (HeLa)	3/3	16	38
CUC-5	1/2	50	13
CUC-9	3/3	35	19
CUC-24	4/4	35	13
CUC-25	4/4	62	8

Legend. Numerator gives number of tumors growing, denominator – number of animals used; *) days after primary injection of material to first transplantation.

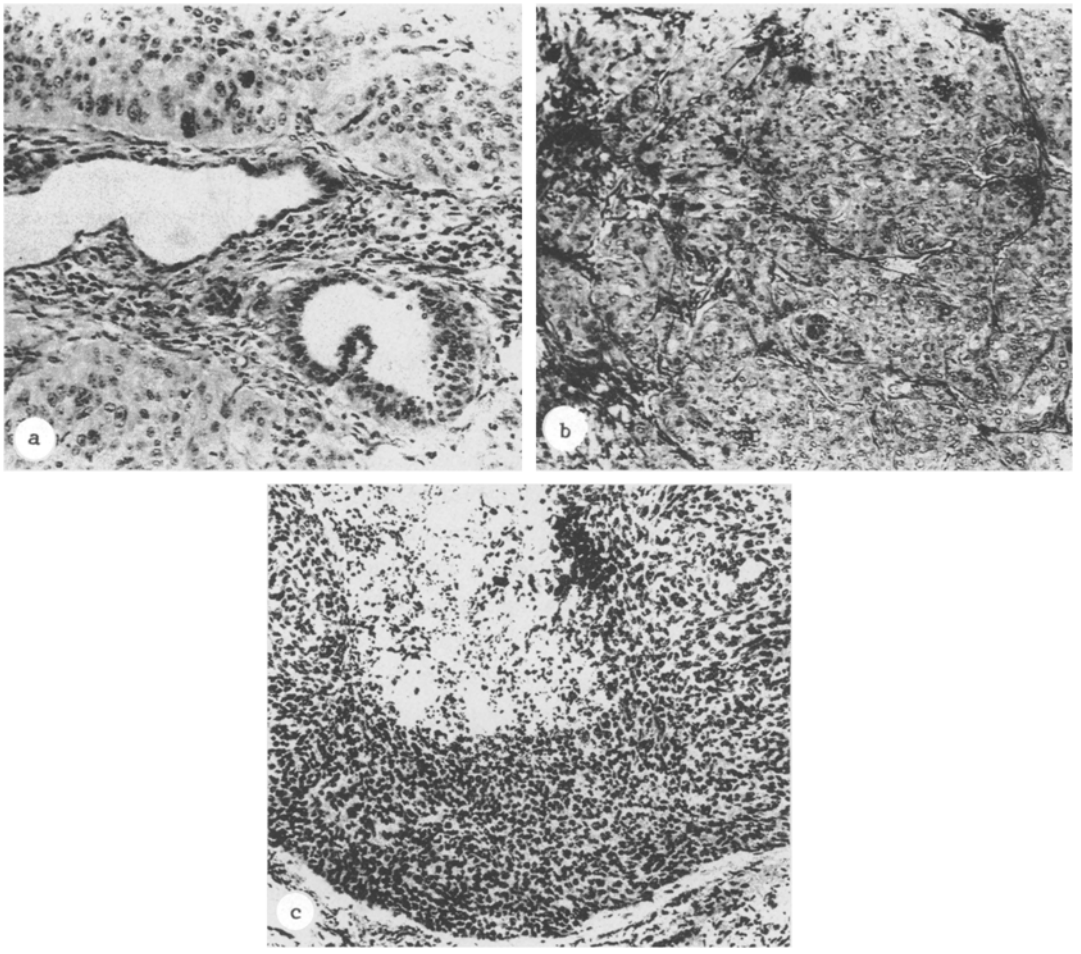

Fig. 1. Histological picture of heterografts of human CUC. Squamous-cell carcinoma: a) material from CUC-9; b) CUC-24; c) CUC-25. Hematoxylin and eosin. 250×.

A histologic investigation was carried out both on material taken from the patients and on tumors in mice at different passages. In all cases the morphological diagnosis was the same – squamous-cell carcinoma (Fig. 1). No histological changes could be found in material obtained from the transplanted tumors compared with material from the original patients, or at different passages of the tumors through the mice.

Molecular Biology. To detect HPV sequences in the cell genome, cellular DNA fractionated in agarose gel and transferred to nitrocellulose, was hybridized with labeled DNA of HPV-16 and HPV-18.

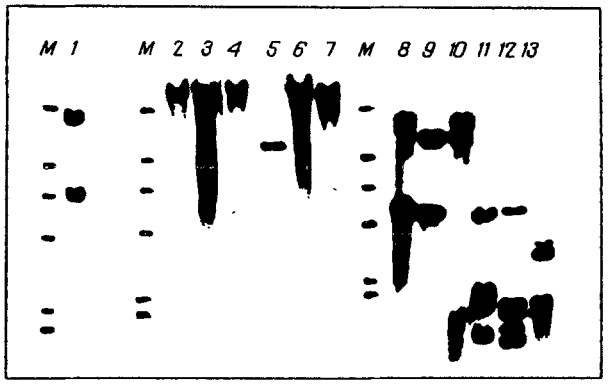

Fig. 2. Blot hybzidization of ^{32}P-DNA of HpV with DNA of CUC xenografts. M) Marker, DNA of phage λ; 1) DNA of CUC-1 (HeLa); 2, 5, 8, 11) DNA of CUC-9; 3, 6, 9, 12) DNA of CUC-24; 4, 7, 10, 13) DNA of CUC-25. M, 5, 6, 7) Restriction for HindIII; 2, 3, 4) without restriction: 1, 8, 9, 10) restriction for BamHI, 11, 12, 13) restriction for PstI. a) Hybridization with ^{32}P-DNA of HPV-18; b) hybridization with ^{32}P-DNA of HPV-16.

It was found that HeLa cells, by injection of which the strain CUC-1 was obtained, contain the HPV-18 genome; the restriction pattern for BamHI (Fig. 2), moreover, agrees with that described in the literature [5]. Under strict conditions, DNA of CUC-5 does not hybridize with DNA from either HPV-16 or HPV-18, but DNA of an HPV complementary to the HPV-16 genome is present in CUC-9, 24, and 25 cells.

The HPV-16 genome is known to measure 7900 base pairs and has one recognition site for BamHI restriction endonuclease, six sites for PstI, but no recognition sites for restriction endonuclease HindIII [5]. This provided the basis for investigating the viral genome in cells of three models containing DNA which hybridized with the DNA of HPV-16.

To study the status of the viral genome in the CUC models, the results of hybridization of cellular DNA, cut by restriction endonuclease HindIII, by other restriction endonucleases, and of uncut DNA were compared. In the case of uncut DNA, hybridization was observed in all three preparations in the region of DNA with high molecular weight, after treatment with HindIII, zones of DNA of CUC-24 and CUC-25 hybridizing with the probe were shifted into the region of somewhat smaller molecular weight, but in DNA of CUC-9 a hybridized fragment measuring about 11,000 base pairs was found (Fig. 2).

Restriction of DNA by BamHI in all three models revealed the presence of two hybridized fragments; in one case their measurements did not agree with that of the HPV-16 genome, namely 7900 base pairs. For CUC-9 and CUC-24, only one fragment measuring more than 7900 base pairs was found in each case, whereas for CUC-25 both fragments were larger than 10,000 base pairs.

The largest fragment excised by PstI from the HPV-16 genome is known to measure 2800 base pairs [5]. However, restriction by PstI revealed fragments close to this size only in DNA of CUC-25. On the other hand, fragments larger than 2800 base pairs were present in DNA of CUC-9 and CUC-24, The dimensions of the set of fragments for PstI, observed in preparations of CUC DNA, differ from that described for the HPV-16 genome.

Thus the DNA of CUC-9, 24, and 25 cells was found to contain DNA of human papillomaviruses, highly homologous with HPV-16 or its deleted genome. The results of restriction analysis of DNA of CUC demonstrate that integration of HPV DNA into the cellular genome takes place in all models of CUC. The presence of the signal in the case of uncut DNA in the high-molecular-weight region only indicates either that the virus genome possesses integrated status or that multiple copy extrachromosomal forms are present. However, displacement of the signal into the region of DNA with lower molecular weight (although still larger than the genome with 7900 base pairs) after treatment with restriction endonuclease HindIII enables only one version of the response to be selected: in all three models of CUC containing DNA complementary to the HPV-1G genome, the viral genome is integrated into the cellular DNA. This is confirmed also by specimens of restriction by other enzymes. The presence of fragments of HPV DNA of high molecular weight in BamHI DNA restriction fragments is also an argument in support of the integrated status of the viral DNA.

The number of fragments discovered in restriction by BamHI (two in each case) suggests that tandem integration of HPV genomes is not present in CUC-9, 24, and 25, for the presence of three fragments, one of which measured about 7900 base pairs, was not found in any of the tumors, as it should have been in the case of a nondefective genome, or one of another size in the case of deletions. Thus the number of integrated HPV genomes in DNA of CUC-9, 24, and 25 is most probably one per cell. However, the possibility of multiple integration by segments of the HPV genome with cell sequences, not less in total size than the sum of the BamHI fragments discovered for each DNA, cannot be excluded from the picture obtained.

The picture of restriction for PstI (a "nonstandard" set of fragments, the presence of high-molecular-weight fragments exceeding 2800 base pairs, and also the fact that the sum of the molecular weights of the fragments in all tumors was greater than the size of the viral genome) confirms the integrative status of the latter and the presence of changes in their structure compared with the classical form.

According to preliminary data, transcription of the viral genome takes place in CUC-9, 24, and 25.

We have thus obtained a collection of transplantable xenografts of human cervical carcinoma, characterized with respect to the presence of genomes of human papillomaviruses. These xenografts will be used to study interaction of the HPV genome with human cells, and in addition, they may be used for experimental research into the diagnosis and treatment of cervical carcinoma.

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