HUMAN CERVICAL CARCINOMA CC-32 XENOGRAFT AND COMPARATIVE FLOW CYTOMETRY OF DNA

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

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The urgent importance of the creation of new models of human tumors, especially carcinoma of the cervix uteri, where a connection with the presence of viral genetic information is presumed, is no longer in debate [4].

In the investigation described below a transplantable xenograft of human uterine cervical carcinoma CC-32, containing DNA of human papillomatosis virus and possessing several other properties, was obtained by transplantation of tumor material into nude mice reared by ourselves.

EXPERIMENTAL METHOD

Transplantation of the tumors into nude mice, preparation of histological sections, and their examination were carried out as described previously [1]. Fragments of tumors measuring about 1 mm^3 for electron microscopy were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, postfixed with osmium tetroxide, and the material was then embedded in a mixture of Epon and Araldite. Ultrathin sections were obtained on the LKB III Ultrotome and examined in the JEM 100 CX electron microscope For flow cytophotometry the cells were treated by the usual method, which we described previously [2]. The results of measurement of DNA content are expressed in ploidy units. Calibration was carried out with the aid of human small lymphocytes, in which the DNA content is 2C. The measurements were made on an ICP II cytophotometer (West Germany). The molecular biological investigation of the tumor DNA was carried out by methods described previously [1].

EXPERIMENTAL RESULTS

The transplanted material was obtained during an operation on a patient aged 24 years with stage IV of the disease. Before the material was taken, the patient had undergone a course of radiotherapy. The patient died a short time later from progression of the underlying disease.

The biological characteristics of growth of the xenograft are given in Table 1.

The human nature of the transplanted tumor was confirmed by the lactate dehydrogenase isozyme composition, consisting of five fractions, predominantly the 3rd and 4th.

The morphological picture of the tumor in this patient was a squamous-cell carcinoma. In the xenograft of the tumor the same morphological picture was observed (Fig. 1a). During passage, no histological changes were found compared with the original material, either depending on the number of transplantations, and no metastases were found in any of the mouse's organs.

Electron-microscopic investigation revealed two varieties of tumor cells: the cytoplasm of one variety contained many free-lying ribosomes, whereas the other was characterized by an abundance of small cytoplasmic vesicles. Junctions in

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TABLE 1. Biological Characteristics of Xenograft CC-32

Primary transplantability	Time of first transplantation, days	Number of passages	Time of transplantations, days	Maximal survival time of mice with tumor, days
3/3*	56	19	18—20	28

Legend. Numerator shows number of tumors growing; denominator – number of animals used.

the form of desmosomes were often seen between the cells (Fig. 1b). Bundles of tonofilaments, characteristic of stratified squamous epithelium, were found in the cytoplasm of some cells (Fig. 1c).

Molecular-biological analysis for the presence of HPV-sequences was carried out with the aid of plasmids containing the genome of types HPV-16 and HPV-18, generously provided by Professor H. zur Hausen (West Germany). On hybridization in the presence of 50% formalin, the CC-32 cells were found to contain DNA complementary to the HPV-16 genome (Fig. 2). The genome of HPV-16 is known to measure 7.9 kbp, it has one recognition site for restriction endonuclease BamHI, two sites for EcdRI, six PstI sites, and no recognition points for restriction endonuclease HindIII [3]. Comparison of hybridization of unrestricted DNA and restricted HindIII showed that the viral DNA is integrated into the cell genome at at least five separate sites. This follows from the fact that the molecular weight of each of the fragments hybridized with HPV-16, and restricted by HindIII, is smaller than in the case of unrestricted DNA, but is greater than 7.9 kbp, i.e., it is longer than the length of the linearized genome of the virus. Restriction of CC-32 DNA by BamHI revealed three major and five minor fragments, hybridized with the total HPV-16 probe. It follows from the restriction map of HPV-16 and the five separate integrative sites which we demonstrated, that we could expect the presence of 10 BamHI fragments. The number we actually found is probably due to superposition of fragments of similar size. Restriction maps of endonucleases PstI and EcoRI, detected by the same probe, are shown. Considering the multiplicity of the integrative sites of the HPV-16 genome in CC-32 DNA, interpretation of these data is difficult.

A comparative cytophotometric investigation of DNA of strain CC-32 and of xenografts of human cervical carcinoma stained by the writers previously [1], was carried out. In all tumors except CC-32 the picture was similar (Fig. 3), indicating that most cells in CC-5, CC-9, CC-24, and CC-25 have a diploid DNA content. In the CC-32 tumor, however, the DNA distribution curve is characterized by a neardiploid peak of DNA content at 2C ± 1/2C. This peak is represented by cells entering the G_1/G_0 phase, and amounts to 35%. The cell population containing 4C ± 1/2C consists of tetraploid cells in the G_1/G_0 phase, and also of diploid cells in the G_2/M phase, and amounts to about 40%. The strain also contains polyploid cells, containing 5C-7C. Compared with other xenografts of aervical carcinoma obtained previously, namely CC-5, CC-9, CC-24, and CC-25, a special feature of the CC-32 model we now describe is that the tumor contains two varieties of cells differing in their DNA content: diploid and tetraploid. The reason for this phenomenon, which distinguishes CC-32 from other xenografts of carcinoma of the cervix uteri is unknown.

Each strain has a characteristic ratio between cell populations that differ in their DNA content. This ratio may even be used to identify the strain. Thus the picture shown in Fig. 3 is a stable characteristic of kinds of human cervical carcinoma CC-5, CC-9, CC-24, CC-25, and CC-32, subjected to serial passage.

Two varieties of cells also can be distinguished in sections of CC-32 by electron microscopy. Consequently, the model obtained is heterogeneous as regards both morphological composition and DNA content, or in other words, the tumor evidently has a multicomponent cellular composition.

Yet another discriminating marker of the cell populations of the tumor may perhaps be the presence or functioning of the human papillomatosis virus genome. We previously obtained xenografts of uterine cervical carcinoma both with the HPV-16 genome (CC-9, CC-24, CC-25) and also in which no DNA of either 16th or 18th types was found (CC-5) [1]. However, only in tumor CC-32 are five separate integration sites found. We know of only one other model system of human cervical carcinoma [5] – cell line QG-H – in which several integration sites of the HPV virus genome have been demonstrated.

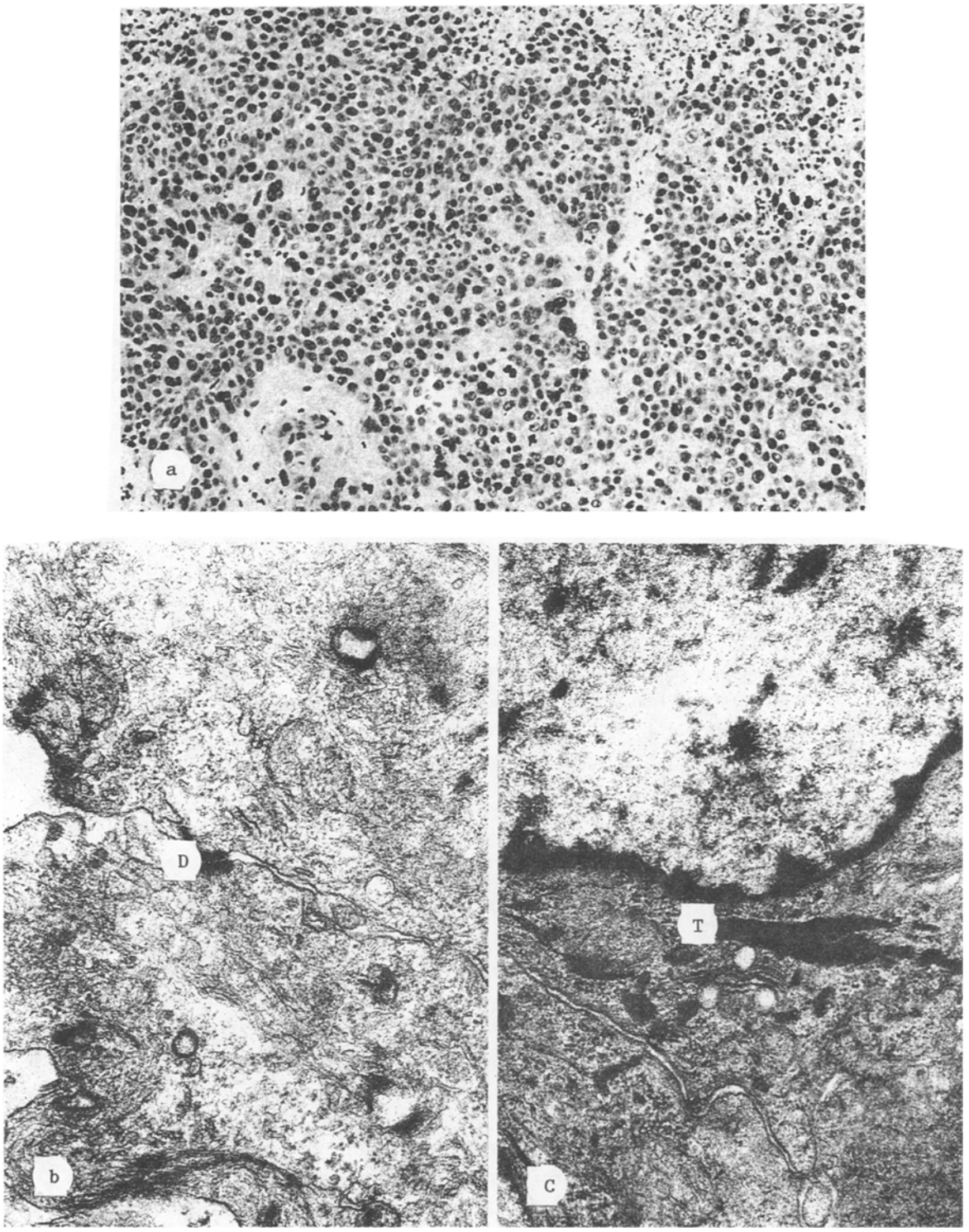

Fig. 1. Histological picture of the CC-32 xenograft: a) squamous-cell carcinoma (hematoxylin and eosin, 160×); b and c) electron-microscopy (stained with osmium tetroxide, magnification 10,000); T) tonofibrils, D) desmosomes.

The model of a tumor with multiple integration sites can be used to investigate the importance of individual sites of insertion of the virus genome for different aspects of cell function.

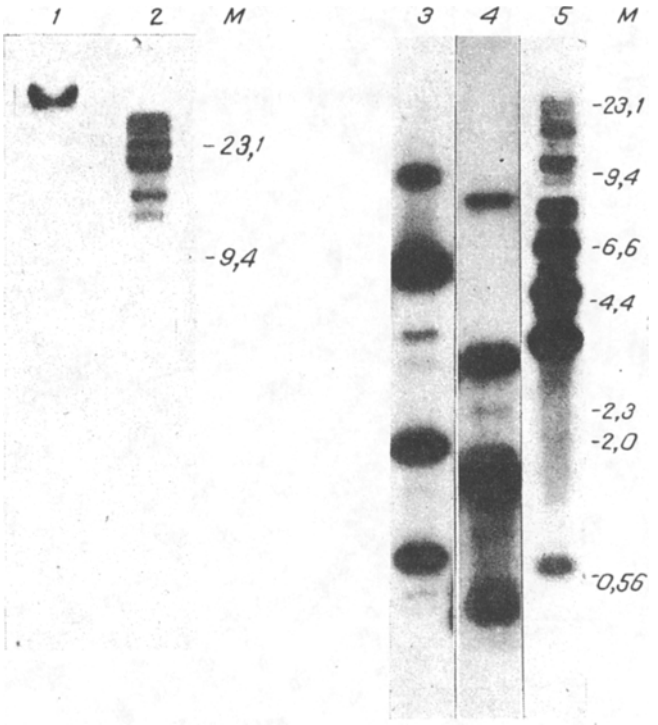

Fig. 2. Hybridization of ^{32}P-DNA of HPV-16 with DNA of CC-32. M) Marker (DNA of phage λ, restricted by HindIII), 1) unrestricted DNA; 2) DNA restricted by HindIII, 3) restriction by BamHI; 4) restriction by PstI; 5) restriction by EcoRI.

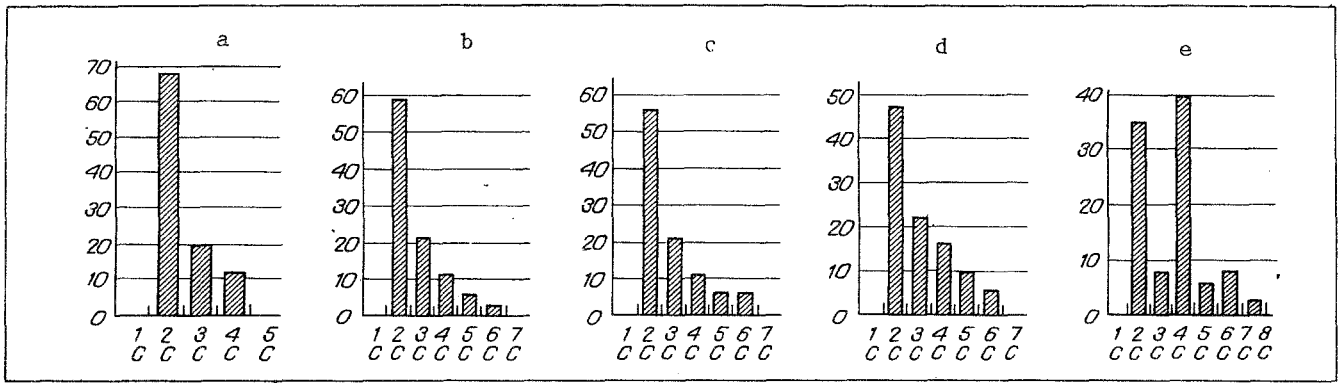

Fig. 3. DNA content in cell populations of cervical carcinoma strains. Abscissa, ploidy, ordinate, number of cells containing the corresponding amount of DNA (in %). a) CC-5; b) CC-9; c) CC-24; d) CC-25; e) CC-32.

Allowing for the presence of several types of markers of cell populations composing a tumor, it is possible to study the dynamics of the cell composition of the tumor and interaction of cell clones in the course of passage or as a result of exposure of the tumor to various influences.

Yet another distinguishing feature of CC-32, as also of other xenografts obtained from clinical material, is that this tumor did not pass through the stage of cell culture, with its specific selection. It has to be pointed out that CC-32, like xenografts of uterine cervical carcinoma obtained previously, spent a limited time in culture in vitro, and it is therefore much more likely to have preserved more of the properties of spontaneous tumors than cell lines such as HeLa, which have

undergone prolonged passage . Naturally CC-32 can be used for research in the experimental diagnosis and treatment of human uterine cervical carcinoma.

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A SIMPLE METHOD OF TESTING SENSITIVITY OF HUMAN TUMORS TO ANTITUMOR AGENTS IN VIVO

A. Lockshin, N. I. Polyanskaya, Yu. V. Mashkovtsev, and K. G. Dzyubanov

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The result of testing hemotherapeutic preparations for their efficacy on human tumors transplanted into nude mice [4] and into mice with immunodepression [11] is in accordance with clinical observations. It has also been found that the treatment of experimental tumors in animals often does not give the same effect in man [10]. To assess the activity of antitumor preparations, the method of implantation of human tumors beneath the renal capsule (RC) of nude mice is used, but the necessity of creating special conditions for the keeping of such animals limits their usefulness. To conduct a similar investigation on mice with temporary immunodeficiency (ID) it is advantageous to use human tumors, such as the nonpigmented melanoma BRO [6], which proliferates rapidly after implantation into nude mice [8] and mice with ID [1, 2, 9]. It has been shown that BRO cells, embedded in a fibrin clot (FC), grow rapidly and remain viable in normal mice previously subjected to whole-body irradiation in a dose of 5.5 or 6.5 Gy [3].

In the investigation described below the conditions for creating optimal ID for growth of human melanoma BRO and for testing the sensitivity of the tumor to cytostatics on this model were determined.

EXPERIMENTAL METHOD

BRO cells were cultured in medium RPMI with 10% embryonic serum and embedded in FC, as described previously [3]. Female (CDF × C57BL/6)F_1 hybrids aged 2-4 months, obtained from the "Stolbovaya" nursery, were used as recipients Whole-body irradiation of the animals was carried out on a ^{137}Cs source (dose rate 0.087 Gy/sec) 24 h before subcapsular transplantation of the tumor. The operation for implantation of FC beneath RC was carried out as described previously [3] and two mutually perpendicular diameters were measured on the day of transplantation and 8 days thereafter, when the mice were killed by cervical dislocation. The antitumor agents were injected intraperitoneally 3 days after

Laboratory of Biochemical Mechanisms of Action of Antitumor Preparations. All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

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