DISCOVERY OF GS-ANTIGEN OF D-TYPE VIRUS IN CELLS OF HUMAN BREAST FIBROADENOMAS

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A combination of immunodiffusion and indirect immunoautoradiographic methods revealed a group-specific (gs-) antigen (or antigens) of a D-type virus, isolated from cells of a continuous culture of human carcinomas and having a common gs-antigen with Mason–Pfizer monkey virus, in five of seven fibroadenomas of the human breast. The results indicate integration of the genome of this or a similar virus with the genome of the fibroadenoma cells. Expression of the virus genome takes place at least as far as the level of synthesis of the virus gs-antigen. The D-type virus from continuous cultures of human carcinoma cells is associated with certain forms of human tumors, one of which is fibroadenoma of the breast.

KEY WORDS: gs-antigen; D-type virus; fibroadenoma of the breast; expression of the virus genome.

The genome of an RNA-containing oncogenic virus (oncornavirus), integrated with the cell genome, is under the control of a different level of repression. The state of subinjection may be characterized by absence of synthesis of virus proteins altogether or by synthesis of certain virus proteins, such as group-specific (gs-) antigens. In the latter case the gs-antigens are specific markers of expression of the oncornavirus genome [11]. The gs-antigen is an internal principal structural protein of oncornaviruses [10] with a molecular weight of about 30,000 daltons [16].

A test system [12] has been obtained against the gs-antigen of a D-type virus isolated from continuous cell cultures of a human carcinoma [4, 5]. A common gs-antigen of D-type virus and of Mason–Pfizer monkey virus (MPMV) has been found [8], and characterizes the group of D-type oncornavirus of primates [6, 12]. Results obtained by the methods of immunofluorescence [14], molecular hybridization [1, 2, 9], and radioimmunoprecipitation [13] methods have shown that the viruses of this group associate with certain human tumors. In a previous communication the writer described having found gs-antigen of D-type virus in two fibroadenomas of the human breast and in a fibroadenoma of the uterus of a patient with carcinoma of the breast [3].

In the investigation described below a search was made for gs-antigen of D-type virus in the cells of fibroadenomas of the human breast by a combination of immunodiffusion and immunoautoradiographic methods.

EXPERIMENTAL METHOD

The method obtaining the immunological test system for gs-antigen of D-type virus and its characteristics were described previously [12].

Antigens of fibroadenomas and carcinomas of the human breast were prepared on the day of removal of the tumors. The minced tumors, added to 0.14 M NaCl solution in the ratio of 1:2, were disintegrated in the "Ultraturrax" homogenizer (Janke and Kunkel, West Germany) for 45 sec. The material was centrifuged at 18,000 g for 20 min. The supernatant was used as the antigen. Antiserum was absorbed by gs-antigen of D-type virus by the following method: Antigen (purified D-type virus, treated with Tween-80 and ether) was put into a well in agar and, after it had diffused into the agar, antiserum was poured into the same well; the antiserum was absorbed as it diffused through the gs-antigen located in the agar.

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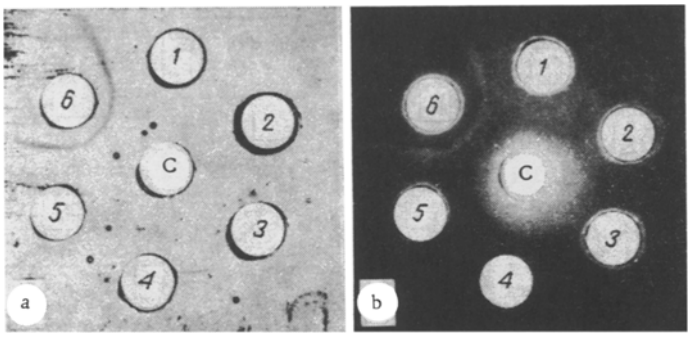

Fig. 1. Discovery of gs-antigen of D-type virus by immunodiffusion (a) and by a combination of immunodiffusion with immunoautoradiography (b). 1) Antigen of fibroadenoma of human breast; 2-4) buffer; 5) gs-antigen of D-type virus; 6) antiserum against gs-antigen of D-type virus; C) antiserum against gs-antigen of D-type virus precipitates antigen of fibroadenoma, as shown by precipitation band identical with precipitation band of gs-antigen of D-type virus. Antiserum absorbed by gs-antigen of D-type virus does not precipitate this antigen or antigen of fibroadenoma.

The immunodiffusion test was carried out in 2% agar. For some tests the indirect immunoautoradiographic method was used [7, 15]. A donkey antirabbit γ-globulin labeled with ^{125}I (commercial preparation obtained from the "Medradiopreparat" factory, Ministry of Health of the USSR), was used.

EXPERIMENTAL RESULTS

Altogether seven fibroadenomas and 26 carcinomas of the human breast were investigated. The gs-antigen (antigens) of D-type virus was found in five fibroadenomas. The results of the immunodiffusion test with and without the use of immunoautoradiography, are shown in Fig. 1. The rabbit antiserum of the test system for gs-antigen of D-type virus precipitated antigens from cells of the fibroadenomas. Precipitation bands were identical with the precipitation band of gs-antigen of the test system. Antiserum absorbed by gs-antigen of D-type virus did not precipitate antigen of the test system or antigen of the fibroadenomas. The gs-antigen of D-type virus absorbed the antiserum completely, for a precipitation band appeared between the wells with the absorbed and unabsorbed antisera. Consequently, antigen in the absorbed antiserum was present in excess. None of the 26 carcinoma antigens reacted with antiserum against D-virus.

It can be concluded from these results that cells of fibroadenomas of the human breast contain gs-antigen of D-type virus isolated from transplantable human carcinoma cells or of MPMV, of a similar oncornavirus possessing a common gs-antigen with these viruses and belonging to the group of D-type oncornaviruses of primates.

The results of the search for this antigen by the immunodiffusion method were conclusive, for the identity of the precipitation bands aroused no doubts about the specificity of the results obtained, something which has to be considered in other immunological methods (immunofluorescence, molecular hybridization, radioimmunoprecipitation). The use of immunoautoradiography makes the method used particularly sensitive.

The following conclusions can be drawn from the results: 1) The genome of D-type virus is integrated with the genome of fibroadenoma cells; 2) the genome of the virus is expressed at least as far as the level at which synthesis of gs-antigen takes place. The mechanism of derepression of the genome is perhaps connected with the function of hormones which participate in the pathogenesis of fibroadenoma of the breast.

The gs-antigen of D-type virus discovered by these experiments is thus a marker of expression of the genome of this (or a similar) virus, integrated into fibroadenoma cells. D-type virus supposedly associates with several forms of human tumors, one of which is fibroadenoma of the breast.

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DIURNAL RHYTHMS OF CELL PROLIFERATION IN LATE PRECANCEROUS CHANGES INDUCED IN THE LIVER BY ORTHOAMINOAZOTOLUENE

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Orthoaminoazotoluene was administered to mice for 9 months. Diurnal rhythms of mitotic activity and of the number of DNA-synthesizing cells were established by autoradiography with ^{3}H-thymidine in stages II (adenomatous nodules) and III (primary hepatomas) of carcinogenesis in the developing tumors and surrounding parenchyma of the liver. A monomodal rhythm of mitotic activity was demonstrated in the structures mentioned above, with the number of mitoses reaching a maximum at 4-7 a.m., whereas the diurnal rhythm of the index of labeled nuclei was bimodal, with maxima at 7 p.m. and 4 a.m. The mean diurnal values of both indices at stages II and III of hepatocarcinogenesis were considerably higher than in the surrounding noncancerous liver tissue.

KEY WORDS: orthoaminoazotoluene; late stages of hepatocarcinogenesis; diurnal rhythms of mitosis and of DNA-synthesizing cells.

An important addition to Founds' concept of tumor progression is represented by the successive morphological stages of precancerous changes in organs and tissues common to different types of carcinogenesis, established by Shabad [9].

There is every reason to suppose that growth which has escaped to some degree from the control of the organism is one of the first stages of progression and is an inseparable property of any tumor [8].

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