STUDY OF MEMBRANE ANTIGENS OF TRANSFORMED AND TUMOR HAMSTER CELLS

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Antigenic simplification of tumor cells was first discovered by Weiler [5] in a primary rat hepatoma. The phenomenon of antigenic simplification has been studied in detail, using mouse hepatomas as the model, by Gel'shtein [2], Abelev et al. [1], and Khramkova and Abelev [3]. By the use of the anaphylaxis with desensitization method, the agar diffusion test, and immunoelectrophoresis the individuality of the antigenic structure of different strains of hepatomas and the development of a process of antigenic reorganization during progression of tumors have been demonstrated. However, normal membrane antigens of living tumor cells have been inadequately studied. Meanwhile, the reasons why tumor cells do not obey signals regulating cell behavior may be a difference in the composition or quantity of normal membrane antigens on the surface of tumor cells compared with normal differentiated cells.

The object of this investigation was to compare the composition of normal membrane antigens of various Syrian hamster continuous tissue culture cells.

EXPERIMENTAL METHOD

Membrane antigens were studied by the mixed hemadsorption test (MHT) as described by Barth et al. [4]. The MHT can be used to study membrane antigens of living, growing cells in a monolayer without disturbance of their contacts with other cells. The following tissue culture lines were used: lines of embryonic hamster cells transformed in vitro by various viruses: HE4 (with bovine adenovirus), HE30 and HE239 (with ts A30 and ts A239 mutants of SV40 virus), HEK40 (with monkey cytomegalovirus); or spontaneously: HETR; lines of hamster tumors: 8/90, E-1, 7L, 20/90 (induced by SV40 virus; the first three, in hamsters of the ICV line), 874 (induced by polyoma virus); cell lines from other species of animals, namely mice, and from man; SV3T3 and M22 (transformed by SV40 virus) and L (transformed by methylcholanthrene); CV-1 (spontaneously transformed green guenon kidney cells); HeLa (human uterine cervical carcinoma cells and primary cultures of hamster embryonic tissue cells (HE). Two rabbit sera obtained as a result of three weekly intramuscular injections of cells of the 8/90 strain in a dose of 5×10^7-40×10^7 into two rabbits, the first time mixed with Freund's complete adjuvant, were used as the immune sera. Blood was taken 20 days after the last injection. To detect cross-reacting antigens the sera were exhausted twice or three times with 50% suspensions of test cells or with a combination of the cells to be investigated.

EXPERIMENTAL RESULTS

To verify the species specificity of the test sera their activity against cells from different species of animals was investigated. The sera reacted effectively with hamster cells of all strains (titers 1:1000-1:20,000) and with mouse cells of strains L and SV3T3 (titers 1:1000-1:3000), and rather less strongly with mouse cells of strain M22, monkey cells of strain CV-1, and human cells of strain HeLa (titers 1:300-1:1000). A series of adsorption experiments showed that mouse, monkey, and human cells reacted with sera against hamster cells mainly on account of interspecific antigens, for HETR and E-1 cells abolished the reaction of the immune sera with cells from animals of other species practically completely. Activity of sera against hamster cells after adsorption with mouse cells of L strain was reduced by only 50-67% to titers of 1:720-1:6400. Consequently, the test sera revealed not only interspecific antigens (described in Table 1 as A1), on hamster cells of the different strains, but also a large group of species-specific antigens.

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TABLE 1. Conventional Scheme of Distribution of Syrian Hamster Membrane Antigens Detected by the MHT with Unexhausted and Adsorbed Immune Heterologous Sera against Hamster Tumor Cells

Strain	Antigen							
	A1	$A2_I$†	$A2_{II}$†	$A2_{III}$†	A3	A4	A5	A6
L	+	—*	—	—	—	—	ND	ND
HE	+	+	+	—	—	—	ND	—*
HEK40	+	+	—	—	—	ND	ND	+
HETR	+	+	—*	—*	—	+	+	+
HE30	+	+	+	—	ND	+	ND	+
HE4	+	+	+	—	+	+	ND	+
HE239	+	+	+	—	+	ND	ND	ND
20/90	+	+	+	+	—*	+	+	+
8/90	+	+	+	+	+	+	+	+
874	+	+	+	+	+	+	—*	+
E-1	+	+	+	+	+	—*	+	ND
7L	+	ND	+	+	ND	ND	ND	ND

Legend. *) Results obtained by complete exhaustion of immune sera by cells of the given strain; †) differences between strains with respect to antigens described as A2 were discovered in experiments with successive combined adsorption of immune sera by cells of normal embryonic hamster tissue and by cells of strain HETR. ND) Not determined.

The multiplicity of the decrease in titer of the sera with respect to all strains of hamster cells was significantly greater (by 5-16 times) after adsorption with cells of strain HETR than after adsorption with mouse cells of strain L. Cells of strain HETR evidently removed not only antibodies against interspecific antigens from the serum, but also antibodies against specific hamster antigens (described in Table 1 as $A2_I$). However, after adsorption of the sera with cells of strain HETR to complete exhaustion against themselves, a strong reaction of the sera still remained (titers 80-640) against nine of the other 10 strains of hamster cells studied. This reaction evidently took place on account of antibodies against antigens not present on HETR cells, for additional (the third) adsorption with HETR cells did not lower the titer of the sera with any of the positively reacting strains.

Since the sera investigated were obtained against cells of a tumor induced by SV40 virus, it was possible that after adsorption with cells of the HETR strain antibodies against tumor antigens specific for SV40 virus would remain in the sera. Evidence against this conclusion was given by the following two facts: 1) the test sera after adsorption with HETR cells lost their activity against mouse cells transformed by SV40 virus; 2) cross-adsorption experiments, with serum adsorbed twice with cells of strain HETR (described as A_X), detected only common antigens in cells of tumors induced by SV40 virus and polyoma virus.

It was further observed that after adsorption with HE cells the activity of serum A_X was reduced against all strains reacting positively with it by 4-8 times or more. Consequently, all these strains had antigens (described as $A2_{II}$) in common with those on HE cells, but absent on cells of strain HETR. It was also found that exhaustion of serum A_X by HE cells led to complete removal of activity of the serum both against normal HE cells and against HE cells transformed in vitro (strains HE4, HE30, and HE239). The serum retained its activity against tumor strains E-1, 20/90, 8/90, 874, and 7L (titers 1:20-1:100). This activity was virtually not reduced as a result of additional adsorption with HE cells. The experiments thus revealed an antigen common to the cells of all five tumor strains studied, but absent in HE cells, whether normal or transformed in vitro (described as $A2_{III}$). The results of subsequent cross-adsorption experiments showed that cells of the continuous tumor strains, irrespective of their origin and line to which they belonged, differed from one another in the composition of their membrane antigens (described as A3, A4, and A5). These experiments also revealed certain additional differences between the tumor strains and the HE cells transformed in vitro. The quantitative differences discovered proved to be stable when reinvestigated.

Another interesting question was whether transformed embryonic cells possess antigens absent in normal embryonic cells, but present in cells of transplantable tumor strains. Such antigens (described as A6) were found in all types of transformed embryonic cells tested.

Quantitative and qualitative differences were thus found in the composition of membrane antigens of transformed and tumor Syrian hamster cells. These differences may evidently reflect the clonal nature of origin of the tumors, the stage of differentiation of the normal cell at which it was fixed by the transformation process, and the disturbance of synthesis of individual antigens by the cell during transformation and progression.

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ULTRACYTOCHEMICAL STUDY OF NUCLEAR NUCLEOSIDE PHOSPHATASE ACTIVITY IN EPITHELIAL CELLS OF THE NORMAL HUMAN GASTRIC MUCOSA AND GASTRIC CARCINOMA CELLS

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The role of adenosine triphosphatase (ATPase) in energy metabolism is well known. The ATP "pool" in the cell is maintained, in particular, by the enzyme inosine diphosphatase (IDPase). The function of this enzyme in the cells has not yet been fully explained. However, some workers [5, 8] consider that inorganic phosphate and inosine are liberated from inosine diphosphate under the influence of IDPase. During intracellular metabolism, adenosine is formed from inosine and is phosphorylated to form ATP.

Most workers [4, 7, 10, 14] describe the localization of the reaction product for ATPase at the light histochemical level entirely in the parietal cells. As regards the electron-histochemical study of ATPase and IDPase in cells of the gastric mucosa, there are only isolated reports [3, 11-13], the authors of which have not paid the necessary attention to the activity of these enzymes in the cell nuclei. Data on the electron-histochemical detection of these enzymes in nuclei of human gastric carcinoma cells are nowhere to be found.

Accordingly, the aim of the present investigation was to compare ATPase and IDPase activity in nuclei of normal epithelial cells of the human gastric mucosa and in gastric carcinoma cells.

EXPERIMENTAL METHOD

Pieces of tissue obtained by means of a fiberoptic gastroscope from tumors of the human stomach and from the gastric mucosa of persons without gastric tumors (control) were studied.* To investigate IDPase and ATPase activity, electron-histochemical methods were used.

*The gastroscopy and taking of the specimens were carried out by Cand. Med. Sci. E. A. Mikhailov (Department of Endoscopy, Oncologic Scientific Center, Academy of Medical Sciences of the USSR), to whom the writers are grateful.

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