A STUDY OF EMBRYONIC α-GLOBULIN AND SERUM PROTEINS OF CULTIVATED MOUSE TUMOR CELLS

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After prolonged culture (3 years) of cells obtained from a solid form of transplantable mouse hepatoma (XXIIa) their ability to synthesize embryonic α-globulin and serum proteins (albumin and transferrin) was shown to be preserved. Malignancy was preserved 3 years after the beginning of cultivation of the hepatoma cells. Embryonic α-globulin was found in the serum of C3HA mice inoculated with hepatoma XXIIa cells after a long period (3 years) in culture.

KEY WORDS: cultivation of tumor cells; embryonic α-globulin; embryo-specific protein; serum proteins.

The ability of tumors of the liver to synthesize embryo-specific α-globulin has been demonstrated and it is a fact of great importance for the immunodiagnosis of cancer [1-3, 10]. Cultivated cells of the ascites form of mouse hepatoma XXIIa in the first weeks of culture were shown to produce embryo-specific α-globulin, the synthesis of which ceased completely during subsequent cultivation, to be renewed when the cells were inoculated in vivo [7, 9]. The study of the presence of embryo-specific and other serum proteins in tumor cells in culture is also interesting for the antigenic marking of tumor cells in culture and for the description of their immunobiologic properties.

The object of the present investigation was to study embryonic α-globulin and serum protein (albumin and transferrin) in cells obtained from the solid form of mouse hepatoma XXIIa and kept for a long period in culture.

EXPERIMENTAL METHOD

Cells of the transplantable line MG XXIIa [4], cells of a clonal culture obtained from the cells of this line by the method described in [5], and also cells of a "zigzag" culture (an explant of a tumor growing in a C3HA mouse after inoculation with cultured cells of line MG XXIIa and passing through 5 generations in them) were used as the objects for the study of embryonic α-globulin and serum proteins. The clonal and zigzag cultures were obtained in the 3rd year of culture of the hepatoma cells. The presence of embryonic α-globulin was also determined in the sera of C3HA mice inoculated with cells of line MG XXIIa, and with the clonal and zigzag cultures. The presence of these proteins in the objects studied was determined by immunoautoradiography [8] and by microprecipitation in agar [6] with the aid of a test system. The presence of synthesis of embryonic α-globulin and of serum proteins (albumin and transferrin) by the cell cultures was determined by immunoautoradiography in growth medium concentrated 8-10 times. Embryonic α-globulin was detected in the sera of the mice with tumors by microprecipitation in agar. The test sys-

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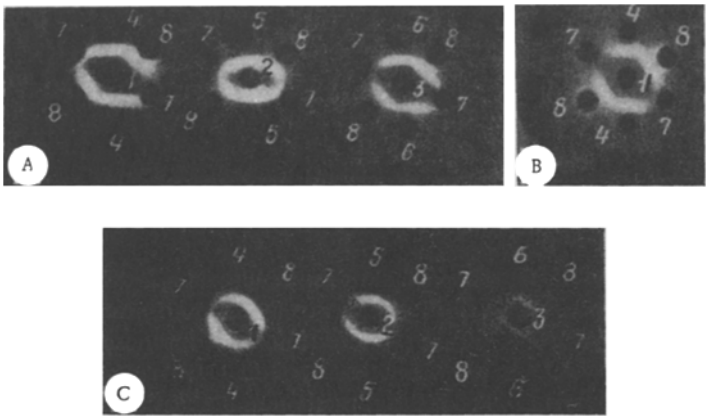

Fig. 1. Determination of embryonic α-globulin, albumin, and transferrin in growth medium of cultures of MG XXIIa tumor cells by immunoautoradiography: A) detection of embryonic α-globulin with test serum for α-globulin; B) determination of albumin by test system for albumin; C) determination of transferrin by test system for transferrin. 1) antiserum of test system in dulution of 1:8; 2) 1:16; 3) 1:32; 4) antigen of test system in dilution of 1:8, 5) 1:16, 6) 1:32; 7) physiological saline; 8) growth medium concentrated with Lifogel.

TABLE 1. Synthesis of α-Fetoprotein, Albumin and Transferrin by Cells of a Solid Form of Hepatoma XXIIa in Culture for Long Periods, and Transplantability

Cell cultures	Period of cultivation (months)	Presence of proteins			Dose of cells inoculated with	Percent of takes
		α-fetoprotein	albumin	transferrin		
Cells of line MG XXIIa	19	+	+	+	10^2—10^6	100
	36	+	+	+	10^2—10^6	100
Clonal culture of MG XXIIa	8	+	+	+	10^2—10^6	100
Zigzag culture of MG XXIIa	30	+	+	+	10^2—10^6	100

tems, generously provided by S. D. Perova,* consisted of monospecific rabbit sera against mouse α-fetoprotein, albumin, and transferrin.

EXPERIMENTAL RESULTS

The presence of synthesis of embryo-specific α-globulin in cells of line MG XXIIa was determined in the 19th month of cultivation of the cells. The results are shown in Fig. 1. Clearly cells of hepatoma XXIIa in culture for a long time retained the ability to synthesis α-fetoprotein. A culture of hepatoma cells in the 19th month also synthesized the normal components of the serum – albumin and transferrin. In the 3rd year of culture cells of line MG XXIIa also continued to synthesize embryonic α-globulin, albumin, and transferrin. In the 3rd year of cultivation clonal and zigzag cultures of cells were obtained and these also retained the ability to synthesize embryonic α-globulin and serum protein – albumin and transferrin.

Cells of the transplantable line MG XXIIa and of the clonal and zigzag cultures were injected into C3HA mice in doses of 10^2-10^6 cells. A 100% take was observed at the same times as the tumors appear when a trypsinized cell suspension of hepatoma XXIIa was injected. This is evidence that mouse hepatoma cells and clonal and zigzag cultures of this hepatoma, kept for long periods in culture, retain their malignancy to a high degree.

Data showing the continued preservation of synthesis of α-fetoprotein, albumin, and transferrin by hepatoma XXIIa cells in culture and the high malignancy of these cells are given in Table 1.

The results of the study of embryo-specific α-globulin in the serum of C3HA mice inoculated with tumor cells of line MG XXIIa kept in culture for long periods showed that the tumor formed in mice after inoculation with these hepatoma cells continued to produce embryonic α-globulin.

As a result of inoculation of BABL/c and C57BL mice with MG XXIIa cells tumors grew which subsequently underwent spontaneous regression. During the period of growth of the tumors, embryo-specific α-globulin was detected in the serum of these mice by microprecipitation in agar.

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The experiments thus showed that cells obtained from a solid form of transplantable mouse hepatoma XXIIa, kept in culture for a long period (3 years), retained their malignancy and their ability to synthesize embryonic α-globulin and serum protein (albumin and transferrin).

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