In the authors' laboratory pararenal angiosarcomas were found in three of five male C3H mice receiving DMH by the same scheme as the CBA mice; there is no information as yet on the appearance of these tumors under the influence of DMH in mice of other lines. There is likewise no information on the mechanisms of the selective appearance of these tumors in male mice; all that can be said is that these tumors did not appear in castrated CBA females.

The development of angiosarcomas of the pararenal cellular tissue under the influence of DMH in male mice is a new and somewhat unusual manifestation of sexual dimorphisms in carcinogenesis, for the tumor concerned is mesenchymal in nature.

LITERATURE CITED

1. K. M. Pozharisskii and V. N. Anisimov, Patol. Fiziol., No. 1, 47 (1975).
2. V. S. Turusov, L. S. Bazlova, and V. A. Krutovskikh, Byull. Éksp. Biol. Med., No. 5, 458 (1979).
3. V. S. Turusov, N. S. Lanko, and L. S. Bazlova, Vopr. Onkol., No. 7, 39 (1977).
4. H. Kirkman, Nat. Cancer Inst. Monogr., 1, 1 (1959).
5. B. Toth, L. Malick, and H. Shimizu, Am. J. Path., 84, 69 (1976).
6. B. Toth and R. B. Wilson, Am. J. Path., 64, 585 (1971).

SYNTHESIS OF α-FETOPROTEIN, ALBUMIN, AND TRANSFERRIN BY CONTINUOUS MOUSE HEPATOMA CELLS

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UDC 616.006-018.1-008.939.6-092.4

The ability of continuous cultures of MGXXIIa mouse hepatoma cells to synthesize α-fetoprotein, albumin, and transferrin was studied by an immunoautoradiographic method. Albumin and transferrin were found in the growth medium of hepatoma cells in the 5th year of culture (55th month), concentrated with polyethylene glycol; no α-fetoprotein could be detected. Only transferrin was found in the growth medium of hepatoma cells in the 8th year of culture (92nd month). Two clonal cultures obtained in the 8th year of culture of hepatoma cells also showed ability to synthesize transferrin. Continuous hepatoma cells preserved their malignancy. In Lyphogel-concentrated sera of mice with tumors formed after inoculation of hepatoma cells in the 5th year of culture, α-fetoprotein was found by the microprecipitation test in agar. No α-fetoprotein was found in the sera of mice with tumors formed after inoculation of hepatoma cells in the 8th year of culture.

KEY WORDS: α-fetoprotein; albumin; transferrin; continuously cultured cells.

The ability of liver tumors to produce α-fetoprotein is utilized for the immunodiagnosis of these tumors [1-3, 9]. Investigation of the synthesis of α-fetoprotein and other serum proteins in cultured hepatoma cells is also of great importance for tagging tumor cells in culture. However, many aspects of this probelm remain unexplained and the literature devoted to its study consists of only isolated communications [5, 7, 8, 10, 11].

The object of this investigation was to study the ability of MGXXIIa mouse hepatoma cells in continuous culture to synthesize α-fetoprotein, albumin, and transferrin.

EXPERIMENTAL METHOD

The MGXXIIa cell line obtained by the writer from a solid form of mouse hepatoma XXIIa [4], and clonal cultures A and B obtained by T. N. Ignatova (Institute of Cytology, Academy

Laboratory of the Molecular Basis of Immunogenesis, Institute of Experimental Biology, Academy of Sciences of the Armenian SSR, Erevan. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 7, pp. 76-78, July, 1979. Original article submitted August 18, 1978.

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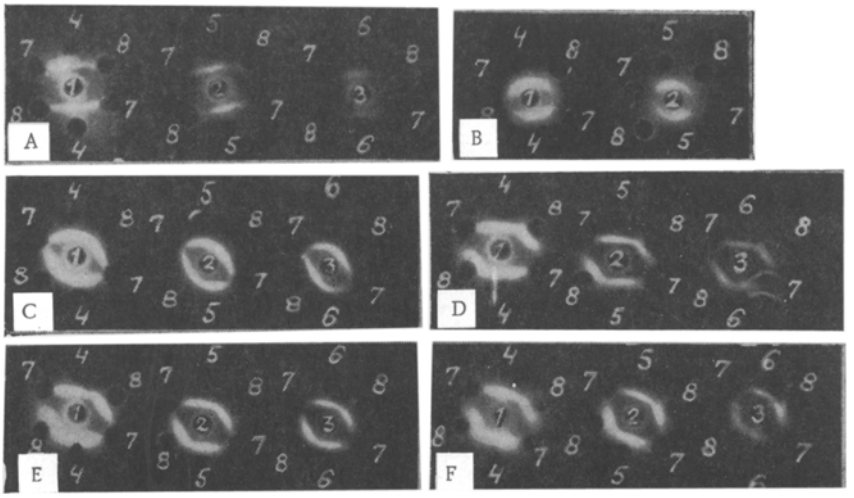

Fig. 1. Detection of α-fetoprotein, albumin, and transferrin in growth medium of continuously cultured mouse hepatoma XXIIa cells by immunoautoradiography. A, B, and C) Detection of α-fetoprotein, albumin, and transferrin respectively at 55th month of cell culture; D) detection of transferrin at 92nd month of cell culture; E and F) detection of transferrin in clonal cultures A and B respectively (8th year of cell culture); 1, 2, 3) antiserum of test system in dilutions of 1:8, 1:16, and 1:32 respectively; 4, 5, 6) antigen of the test system in dilutions of 1:8, 1:16, and 1:32, respectively; 7) physiological saline; 8) growth medium concentrated with the aid of polyethylene glycol.

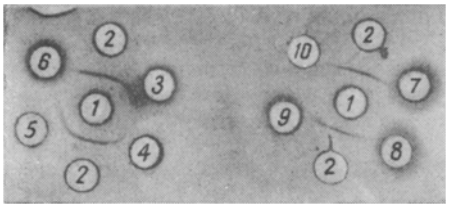

Fig. 2. Determination of α-fetoprotein by the microprecipitation test in agar in sera of mice with tumors formed after inoculation with continuous cultures of mouse hepatoma XXIIa cells. 1) Antiserum of test system in dilution of 1:4; 2) antigen of test system in dilution of 1:4; 3, 4, 5) sera of mice inoculated with hepatoma cells in the 5th year of culture (55th month), concentrated with Lyphogel; 6, 7, 8, 9) sera of mice inoculated with hepatoma cells in eighth year of culture (92nd month), concentrated with Lyphogel; 10) physiological saline.

of Sciences of the USSR) from MGXXIIa cells in the 8th year of culture, were used as objects for the study of α-fetoprotein, albumin, and transferrin. The nutrient medium for cell culture consisted of 80% medium No. 199 and 20% bovine serum. The presence of α-fetoprotein, albumin, and transferrin was determined by the indirect immunoautoradiographic method [6], using test systems, in the growth media of hepatoma cells in the 5th year (55th month) and 8th year (92nd month) of culture and of A and B clonal cultures. The growth media were concentrated 20 times with polyethylene glycol. Antisera of the test systems, generously pro-

vided by A. K. Yazova (Laboratory of Immunochemistry of Tumors, Oncologic Scientific Center, Academy of Medical Sciences of the USSR), consisted of monospecific rabbit sera against mouse α-fetoprotein, albumin, and transferrin. The presence of α-fetoprotein in the sera of C3HA mice, inoculated with continuous hepatoma cells, was studied by the microprecipitation test in agar, with the aid of a test system. The sera of the mice with tumors were concentrated 2-5 times with Lyphogel.*

EXPERIMENTAL RESULTS

The results of the study of α-fetoprotein, albumin, and transferrin in the growth medium of continuous XXIIa hepatoma cells are illustrated in Fig. 1. They show that albumin and transferrin were found in growth medium of hepatoma cells in the fifth year in culture, but no α-fetoprotein could be detected. In the eighth year of culture the hepatoma cells continued to synthesize transferrin only. This protein also was found in growth medium of clonal cultures A and B ,eighth year of continuous culture of hepatoma cells). In the eighth year of culture the hepatoma cells synthesized neither α-fetoprotein nor albumin.

Continuuously cultured cells were inoculated into C3HA mice in doses of 10^5 to 10^6. Hepatoma cells at the fifth and eighth years of culture formed tumors in the experimental animals. This shows that mouse hepatoma XXIIa cells cultured continuously in vitro for a long time preserve their malignancy.

The results of a study of α-fetoprotein in the sera of mice with tumors formed after inoculation of cultured hepatoma cells are given in Fig. 2. Clearly α-fetoprotein was present in the sera of mice with tumors formed after inoculation of hepatoma cells in the fifth year of culture. Meanwhile, no α-fetoprotein could be detected in the sera of mice inoculated with hepatoma cells in the eighth year of culture. These observations show that until the fifth year of culture, culture loss of the ability of the heptoma cells to synthesizeα-fetoprotein was reversible in character, but at the eighth year in culture this ability was evidently lost forever. The results confirm those obtained by Irlin et al. [8], but at the same time, they show that ability of continuously cultured mouse hepatoma cells to synthesize α-fetoprotein disappears completely.

It can thus be concluded from these experiments that the ability of continuously cultured mouse hepatoma XXIIa cells to synthesize transferrin is preserved unchanged, and this feature can be used as a marker of these cells.

LITERATURE CITED

1. G. I. Abelev, Vestn. Akad. Med. Nauk SSSR, No. 7, 49 (1970).
2. G. I. Abelev, Vestn. Akad. Med. Nauk SSSR, No. 2, 23 (1974).
3. G. I. Abelev, S. D. Perova, N. I. Khramkova, et al., Biokhimiya, 28, 625 (1963).
4. Yu. T. Aleksanyan, M. E. Basmadzhyan, K. S. Movsesyan, et al., Byull. Éksp. Biol. Med., No. 5, 94 (1972).
5. M. E. Basmadzhyan and Yu. T. Aleksanyan, Byull. Éksp. Biol. Med., No. 10, 84 (1974).
6. D. A. El'gort and G. I. Abelev, Byull. Éksp. Biol. Med., No. 2, 118 (1971).
7. E. W. Hull, R. W. O'Hara, C. F. Smith, et al., Proc. Am. Assn. Cancer Res., 10, 41 (1969).
8. I. S. Irlin, S. D. Perova, and G. I. Abelev, Int. J. Cancer, 1, 337 (1966).
9. G. O'Conor, Yu. S. Tatarinov, G. I. Abelev, et al., Cancer (Philadelphia), 25, 1091 (1970).
10. S. Quelin, M. Rioche, Y. Bresson, et al., C.R. Acad. Sci. (Paris), L-274, 768 (1972).
11. S. Sell, F. F. Becker, H. L. Leffert, et al., Cancer Res., 36, 4239 (1976).

*The work of determination of α-fetoprotein, albumin, and transferrin in the growth medium of the cultured hepatoma cells was carried out in the Laboratory of Immunochemistry of Tumors, Oncologic Scientific Center, Academy of Medical Sciences of the USSR. The author is grateful to Professor G. I. Abelev, Dr. Med. Sci. I. S. Irlin, and Cand. Biol. Sci. A. K. Yazova for help with this part of the investigation.