BIOSYNTHESIS OF EMBRYONIC PREALBUMIN BY CULTURED HUMAN FIBROBLASTS

M. Yu. Vasil'ev, S. G. Vorsanova,
V. V. Kalashnikov, and Yu. S. Tatarinov

UDC 612.64'398.131

Embryonic prealbumin (EPA) was found in human fibroblasts by immunodiffusion and immunofluorescence analysis. No quantitative or qualitative differences in the content and localization of this antigen were found between embryonic and adult human fibroblasts. It is concluded that human fibroblasts synthesize EPA, which can be used as a marker for cells of this type.

KEY WORDS: embryonic antigens; biosynthesis; human fibroblasts.

Embryonic prealbumin (EPA) was first identified in human fetal blood serum and in tissue extracts from human ovarian tumors [1, 3]. EPA was subsequently found also in tissue extracts of tumors of bone and soft tissues and in extracts of human fibroblasts [4]. This antigen is a sulfated glycoprotein containing about 20% of carbohydrates [2], and it moves on electrophoresis in the zone of prealbumins and albumins.

This paper gives the results of an immunochemical and immunofluorescence study of EPA in embryonic and adult human fibroblasts cultured in vitro.

EXPERIMENTAL METHOD

Antisera against EPA were obtained by immunizing rabbits by the method described previously [1]. Specific antibodies against EPA were isolated on an immunosorbent prepared by immobilization of a purified EPA preparation on "Ultrogel AcA-34" (from LKB, Sweden) with the aid of glutaraldehyde [7]. Immunodiffusion analysis with a standard test system for EPA was carried out in 1% agar gel by Ouchterlony's method with certain modifications [5]. Cultures of fibroblasts were obtained from pieces of skin taken from healthy adult donors and from 8-24-week human embryos and fetuses obtained during artificial termination of pregnancy in Moscow maternity homes, and from amniotic fluid obtained during aminocentesis at the 14th week of pregnancy. The cells were cultured in Eagle's basic medium with the addition of 20% bovine serum and 30% lactalbumin hydrolysate. A homogenate of fibroblasts removed from the culture vessels with 0.25% trypsin solution was washed three times in 0.14 M NaCl, the number of cells was counted in a Goryaev's chamber, and the cells were sedimented by centrifugation at 800g for 5 min. The residue was treated with 0.05 M Tris-glycine extracting buffer (pH 8.3), containing 1% Tween-80 and 1% Triton X-100, up to a final volume of 1 ml, and the resulting suspension was homogenized in a Potter's homogenizer. After freezing in liquid nitrogen and thawing three times, the homogenate was centrifuged at 3500g for 30 min and the supernatant was used in the subsequent experiments.

Immunofluorescence analysis was carried out on a monolayer of human fibroblasts grown on coverslips, containing about 30,000 cells/cm^2 of glass, fixed with a mixture of methanol and acetic acid (3:1), by an indirect immunofluorescence technique [13]. Specific rabbit antibodies against EPA were used as the first-order antibodies, and a luminescent donkey antiserum against rabbit γ-globulins as the second-order antibodies. The specificity of fluorescence was determined with the aid of the following controls: a) treatment of the cells with nonimmune rabbit serum and labeled second-order antibodies, b) treatment of the cells with antibodies against EPA, neutralized with a purified preparation of EPA, and with labeled second-order antibodies, c) treatment of the cells with labeled second-order antibodies only. The protein concentration in the extracts of fibroblasts was determined by Lowry's method [9].

Department of Biochemistry and Problem Laboratory for Immunochemistry of Malignant and Embryonic Tissues, N. I. Pirogov Second Moscow Medical Institute. Laboratory of General Cytogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 2, pp. 176-179, February, 1980. Original article submitted April 16, 1979.

0007-4888/80/8902-0153$07.50

TABLE 1. Immunochemical Determination of Embryonic Prealbumin (EPA) in Extracts of Different Lines of Cultured Human Cells

Origin and type of cells	Sex	Number of cells ($\times 10^6$ in 1 ml extract)	Passage	Titer of EPA	Protein concentration in extract, mg/ml
Embryonic fibroblasts obtained at medical abortion:					
8 weeks	M	250	12	1:8	13,6
12 »	F	90	14	1:4	4,8
14 »	F	60	8	1:2	3,8
14 »	M	60	9	1:2	3,4
24 »	F	50	6	1:2	3,4
16 »	F	35	6	1:2	2,1
Fibroblasts from donor	—	60	5	1:2	3,6
22 years	F	100	10	1:4	5,2
30 »	F	60	6	1:2	3,6
27 »	M	60	7	1:2	3,4
27 »	M	65	7	1:2	3,8
30 »	M	60	9	1:2	3,5
16 »	M	50	9	1:2	3,1
27 »	M	70	8	1:2	3,8
27 »	F	30	6	1:1	1,8
25 »	F	20	8	1:1	1,1
Postnatal fibroblasts obtained from child aged:					
2 years	M	20	8	1:1	1,3
4 »	F	40	6	1:2	2,4
L-66 fibroblasts: embryonic lung	—	100	33	1:4	5,6
HeLa cells: human uterine cervical carcinoma	F	100	—	0	5,4

Legend. –) Data unknown.

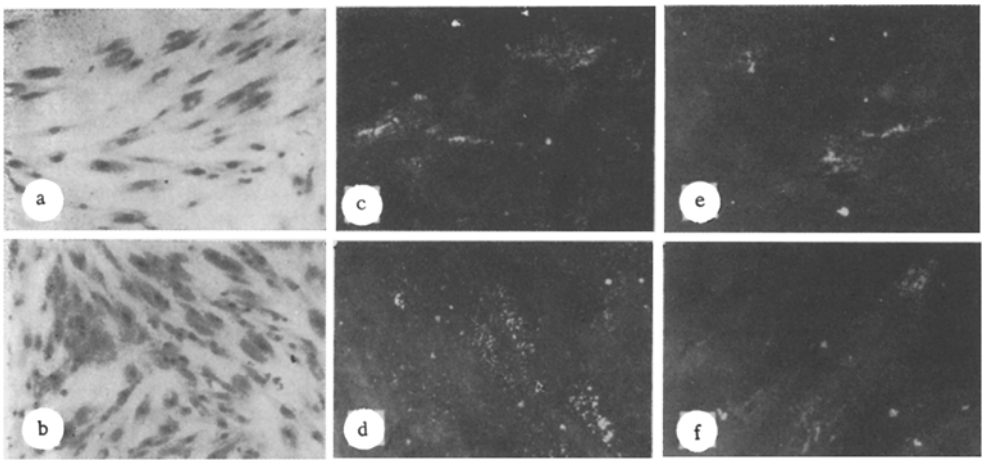

Fig. 1. Immunofluorescence study of embryonic prealbumin (EPA in human fibroblasts: a) monolayer of embryonic fibroblasts, hematoxylin–eosin, 30×; b) monolayer of adult human fibroblasts, hematoxylin–eosin, 30×; c, e) immunofluorescence of EPA on monolayer of embryonic fibroblasts, 120×; d, f) immunofluorescence of EPA on monolayer of adult human fibroblasts, 120×.

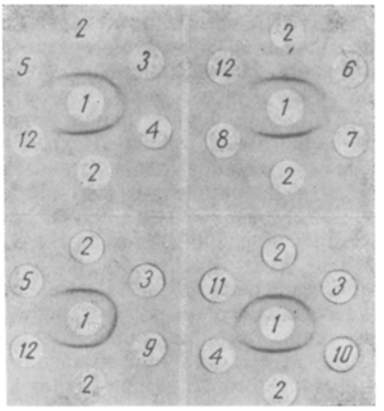

Fig. 2. Immunochemical identification of embryonic prealbumin (EPA) in extracts of human fibroblasts, fetal blood serum, and homogenates of certain human tumors. 1) Specific antiserum against EPA; 2) standard EPA antigen; 3, 4, 5, 6, 7) extracts of human embryonic fibroblasts, adult human fibroblasts, fibroblasts from a child aged 4 years, amniotic fluid, and embryonic lung (L-66), containing 50×10^6, 60×10^6, 20×10^6, 60×10^6, and 100×10^6 cells respectively in 1 ml; 8) extract of HeLa cells (100×10^6 cells in 1 ml); 9) fetal blood serum at 24th week of pregnancy; 10, 11) homogenates of desmoid tumor and osteogenic sarcoma; 12) physiological saline (control).

EXPERIMENTAL RESULTS

All cells of normal diploid lines (Nos. 1-18, see Table 1) of embryonic and postnatal origin possessed the characteristic features of normal human fibroblast-like cells (Fig. 1a, b).

Immunodiffusion analysis with a standard test system for EPA revealed an antigen immunochemically identical with EPA in all extracts from the lines of embryonic and adult human fibroblasts obtained (Table 1). The EPA found in the fibroblasts was immunochemically similar to EPA from human fetal blood serum and also to EPA from homogenates of bone and soft-tissue tumors (Fig. 2). The minimal number of fibroblasts required to detect EPA in these experiments was 20×10^6 cells in 1 ml of extract. Considering the sensitivity of the standard test system (the visible "bending" of the precipitation arc corresponds to an antigen concentration of 1-3 μg/ml), the EPA concentration in the extracts of fibroblasts was only 1/1000-1/2000 of the total protein content in the extract. The EPA titer in the fibroblasts was independent of the sex and age of the source of cells and also of the number of passages of the cell line, and was determined purely by the number of cells taken in order to prepare the extract. No quantitative or qualitative difference in the EPA content likewise could be detected in extracts of embryonic and adult human fibroblasts. A line of fibroblasts from embryonic lung (L-66), generously provided by the Moscow Research Institute of Virus Preparations, also contained EPA. Meanwhile, during immunodiffusion analysis with a standard test system, no EPA could be found in extract of HeLa cells (100×10^6 cells in 1 ml).

On immunofluorescence analysis specific fluorescence of EPA was found in the cytoplasm of human fibroblasts mainly at the ends of the cell processes, in the form of small round granules, and also in the extracellular space (Fig. 1c-f). This localization of the EPA in the human fibroblasts suggests that this antigen is synthesized in the cytoplasm of the cells, from which it is secreted into the intercellular space. However, no difference as regards either the distribution or the intensity of fluorescence could be found between embryonic and adult human fibroblasts. No fluorescence was present on the control preparations. Meanwhile EPA is evidently not identical with fibronectin, the principal high-molecular-weight glycoprotein synthesized by fibroblasts [10, 11]. Unlike that antigen, EPA cannot be found in the blood serum of healthy adult donors by means of the standard test system and it has a much smaller molecular weight. Furthermore, during immunofluorescence analysis, fibronectin in human fibroblasts is located mainly in the extracellular matrix in the form of strands forming a reticular structure [6, 8, 12].

It can thus be concluded from the discovery of EPA in extracts of human fibroblasts cultured in vitro, by means of immunodiffusion and immunofluorescence analysis, that biosynthesis of this antigen takes place in this type of cell. The discovery of EPA synthesis in human fibroblasts enables the appearance of this antigen in tumors of connective tissue origin to be linked with the intensified proliferation of fibroblast-like cells

that are components of these tumors. The presence of EPA in cell lines of fibroblast origin and its absence in a strain of epithelioid cells (HeLa) means that this antigen can be used as a marker of human fibroblast-like cells, and also that these cells can be used as a model for the study of the biological function of EPA.

LITERATURE CITED

1. V. V. Kalashnikov, S. A. Borisenko, and Yu. S. Tatarinov, Byull. Éksp. Biol. Med., No. 6, 726 (1976).
2. Yu. S. Ovodov, V. V. Kalashnikov, A. V. Kurika, et al., Bioorg. Khim., 4, 813 (1978).
3. Yu. S. Tatarinov and V. V. Kalashnikov, Nature 265, 638 (1977).
4. Yu. S. Tatarinov, V. V. Kalashnikov, M. Yu. Vasil'ev, et al., Lancet, 2, 1122 (1978).
5. N. I. Khramkova and G. I. Abelev, Byull. Éksp. Biol. Med., No. 12, 107 (1961).
6. P. Bornstein and J. F. Ash, Proc. Natl. Acad. Sci. USA, 74, 2484 (1977).
7. G. -L. Guesdon and S. Avrameas, J. Immunol. Meth., 11, 129 (1976).
8. K. Hedman, A. Vaheri, and J. Wartiovaara, J. Cell Biol., 76, 748 (1978).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
10. M. W. Mosesson, A. B. Chen, and R. M. Huseby, Biochim. Biophys. Acta, 386, 509 (1975).
11. E. Ruoslahti, A. Vaheri, P. Kuusela, et al., Biochim. Biophys. Acta, 322, 352 (1973).
12. A. Vaheri and E. Ruoslahti, J. Exp. Med., 142, 530 (1975).
13. T. H. Weller, Proc. Soc. Exp. Biol. (New York), 86, 789 (1954).

DEPENDENCE OF MITOGENETIC RADIATION AND UNBALANCED MOLECULAR ORGANIZATION OF LIVER CELLS ON VAGUS NERVE STIMULATION

A. A. Gurvich and T. N. Livanova

UDC 612.35.014.483-06: 612.819.913

Structural changes in the molecular substrate of the rabbit liver arising in vivo in response to weak (subthreshold) electrical stimulation of one trunk of the vagus nerve in the cervical region are examined. The method of investigation was to study the mitogenetic radiation of the liver. It was shown that the degree of saturation of the liver with unbalanced molecular structures (constellations) increases during subthreshold stimulation. The possible mechanism of this relationship is discussed.

KEY WORDS: mitogenetic degradation radiation; unbalanced molecular constellations; vector biological field.

Previous investigations showed that electrical stimulation of the vagus nerve in rabbits increases the number of unbalanced molecular structures or "constellations" (UMC) in the neuroplasm of the contralateral nerve and in the sarcoplasm of heart muscle. An indicator of UMC is degradation mitogenetic radiation, which arises during structural changes and disturbances of UMC.

The study of this dependence is of definite interest in the liver, an organ innervated by branches of the vagus nerve, and of different structure and function, about whose radiation much is already known.

EXPERIMENTAL METHOD

A short segment of the vagus nerve in the neck and a small area (1.5-2 cm^2) of the surface of the liver were exposed in an unanesthetized rabbit. Electrodes were applied to the exposed segment of the nerve and it was stimulated by means of a stimulator. Pulses 1 msec in duration, with a frequency of 40 Hz and an in-

Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 2, pp. 179-180, February, 1980. Original article submitted January 19, 1979.

0007-4888/80/8902-0156$07.50