BIOSYNTHESIS AND INTRACELLULAR LOCALIZATION OF EMBRYONIC PREALBUMIN IN HUMAN FETAL TISSUES

V. V. Kalashnikov, M. Yu. Vasil'ev,
D. M. Falaleeva, T. N. Panina,
and Yu. S. Tatarinov

UDC 612.647.015.348

Immunofluorescence analysis of sections through the chorion and various embryonic tissues was used to determine the localization of embryonic prealbumin (EPA) in cells of the chorionic mesenchyme, bones, umbilical cord, and skin and in the cytoplasm of the epithelium of the distal portion of the renal tubules of the fetus. Synthesis of the slow EPA peak was demonstrated in a suspension culture of chorion, umbilical cord, and bone tissue. It can thus be postulated that EPA is a product of mesenchyme and that evidently it is actively produced during embryonic development. Synthesis of this protein is renewed in tumor tissues. In tumors of connective-tissue origin EPA is localized in the tumor cells. However, tumor cells in tumors of non-connective-tissue origin did not contain EPA, but it was found in the underlying connective tissue. The phenomenon of embryonic reversion probably affects not only "true" tumor cells, but also the surrounding connective tissue.

KEY WORDS: embryonic prealbumin; mesenchymal antigen; desmoid; adenocarcinoma of the ovary; connective tissue.

A hitherto unknown antigen, present in all fetal blood sera and also found in tumor extracts, was identified in 1976 [1, 5, 6]. It possessed the electrophoretic mobility of prealbumin and consequently was called embryonic prealbumin (EPA).

The object of this investigation was to discover the site of synthesis of EPA and to determine its intracellular localization in the fetal tissues and in certain human tumors.

METHODS

Antisera were obtained in rabbits by the use of a semipurified preparation of EPA for immunization [1]. Immunochemical analysis of the resulting antisera was carried out with the aid of a standard test system for this antigen.

To study the cellular localization, the indirect method of immunofluorescence analysis was used on tissue sections obtained after fixation of 6-10-week human embryos, chorion, and certain tumors (desmoid, adenocarcinoma of the ovary), fixed in ethanol and acetic acid [8] and embedded in paraffin wax [10].

The embryonic material was obtained from Moscow maternity homes during medical abortions at 6-10 weeks of pregnancy. The tumor material was obtained during operations at the Oncologic Scientific Center, Academy of Medical Sciences of the USSR.

Antibodies against EPA were isolated from monospecific antisera on an immunosorbent prepared from Ultragel ASA-34 with the aid of glutaraldehyde [7], on which the purified EPA preparation was immobilized. Hyperimmune donkey antisera against rabbit γ-globulins, labeled with fluorescein isothiocyanate,* were used as "second" antibodies.

The specificity of immunoflorescence was determined by means of the following controls: a) treatment of the sections with donkey antiserum against rabbit γ-globulin, labeled with

*The antisera were prepared by the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow.

Department of Biochemistry and Problem Laboratory for Immunochemistry of Malignant and Embryonic Tissues, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 11, pp. 543-545, November, 1979. Original article submitted April 4, 1979.

0007-4888/79/8811-1271$07.50

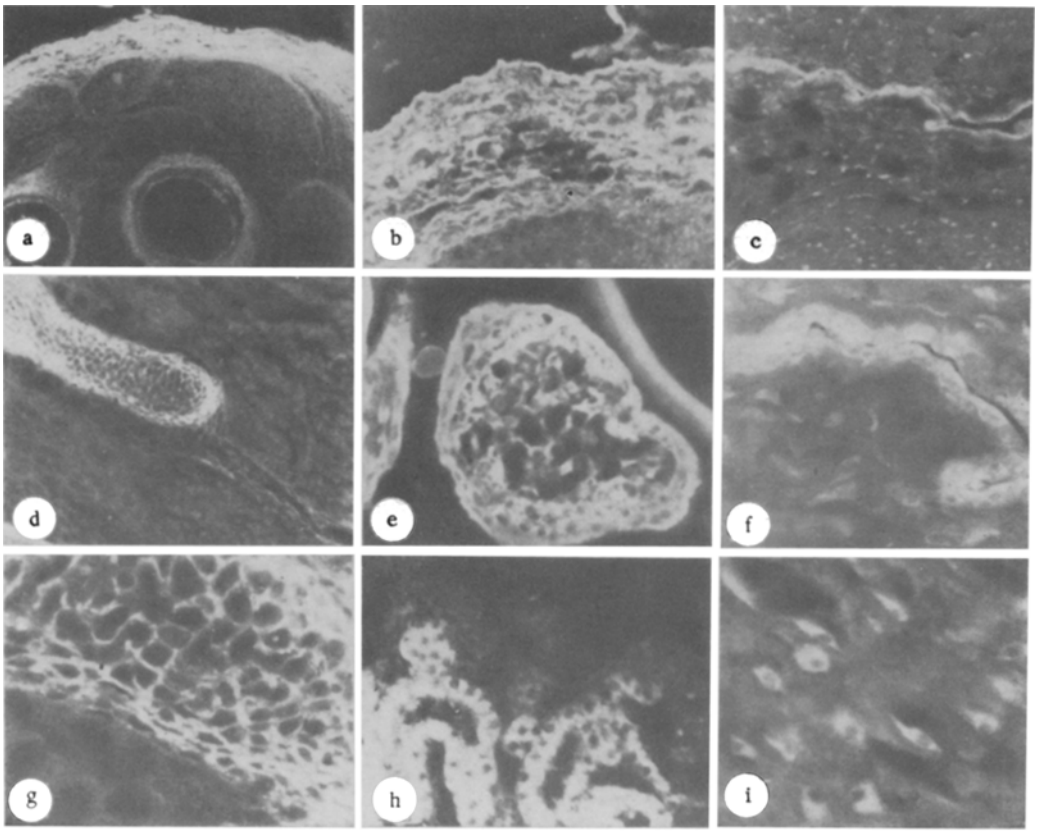

Fig. 1. Immunofluorescence analysis of EPA in sections through chorion and various tissues of 8-week human embryo. a, b) Transverse section through arm of fetus; c, f, i) section through umbilical cord; d, g) longitudinal section through anlage of long bone; e) section through chorion; h) section through kidney. a, c, d) 30 ×; b, e, f, g, h, i) 120 ×.

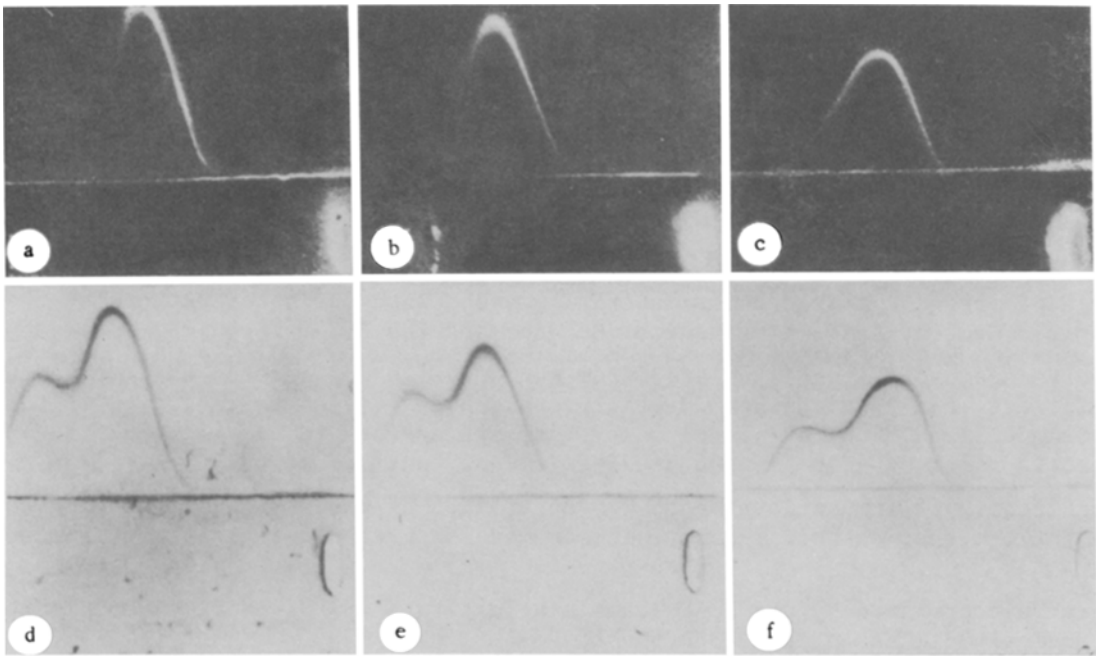

Fig. 2. Radioimmunoelectrophoretic analysis of EPA biosynthesis in suspension culture of bones (a, d), chorion (b, e), and umbilical cord of human fetus (c, f) at 6th-10th week of embryogenesis.

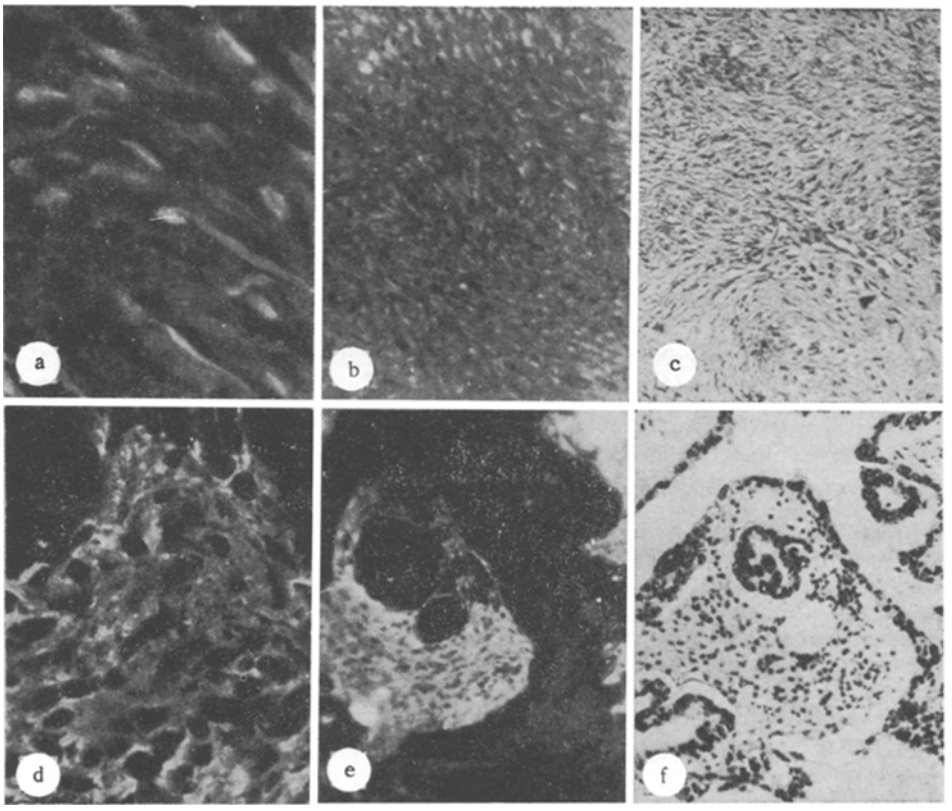

Fig. 3. Immunofluorescence analysis of EPA in sections of desmoid (a, b, c) and adenocarcinoma of the ovary (d, e, f). Hematoxylin—eosin. a, c, e) 30 ×; b, d, f) 120 ×.

fluorescein isothiocyanate alone; b) treatment of the sections with antibodies against EPA after their exhaustion with a purified EPA preparation.

EPA biosynthesis was studied in a suspension culture of various human embryonic tissues by determining incorporation of labeled ^{14}C-amino acid into the protein molecule [4]. Incorporation of label was recorded by radioimmunoelectrophoresis [9] of cultural samples of the chorion (two specimens), embryonic bones (four specimens), liver, kidney, lung, gastrointestinal tract, umbilical cord, and brain (two specimens from each).

RESULTS

Immunofluorescence analysis of sections through the chorion and various embryonic tissues revealed specific fluorescence of EPA-containing structures in the chorion (Fig. 1e), bone tissue (Fig. 1a, d, g), cytoplasm of the epithelium of the distal portion of the renal tubules (Fig. 1h), the skin (Fig. 1a, b), and umbilical cord of the fetus (Fig. 1c, f, i). Maximal fluorescence, moreover, was observed in the chorion, the periosteal part of the bones, skin, and umbilicus. Fluorescence of the epithelium of the distal portion of the renal tubules was weaker.

In the chorion EPA was localized mainly in the cells of the extraembryonic mesenchyme, the basal membrane of the villi, and also in the endothelium and perivascular part of the vessels within the villi. The syncytio- and cytotrophoblast gave weak and diffuse fluorescence.

In the bones EPA was localized in cells of the bony mesenchyme whereas no specific fluorescence was observed in cartilage. The largest number of EPA-containing cells was found in the cranial bones and the bony tissue of the vertebral column. In the long bones, EPA-containing cells were located only in the periosteal part.

By immunofluorescence analysis of sections of the umbilical cord and skin, EPA was found in cells of the mesenchyme, the membrane of the cord, and the vascular endothelium. Fluorescence was absent in the control sections, as it also was in sections of the liver, brain, gastrointestinal tract, lung, and other organs of the fetus.

A study of EPA synthesis in the tissues of the fetus and chorion with the aid of labeled amino acids showed incorporation of the label in a suspension culture of the chorion (Fig. 2b, e), the bones (Fig. 2a, d), and umbilical cord (Fig. 2c, f). No incorporation of label was observed in cultures of the other organs.

The histomorphological structures containing EPA in the chorion, bones, skin, and umbilical cord were thus probably responsible for synthesis of this protein in the fetal tissues. The presence of EPA in the cytoplasm of the epithelium of the distal portion of the renal tubules could be explained either by synthesis or by reabsorption of the primary urine of the fetus, in which EPA is present, by this part of the tubule. However, not all EPA, but only part of it, is reabsorbed from the primary urine. The rest is excreted with the urine into the amniotic fluid, in which EPA also is present [2]. The presence of EPA in the amniotic fluid could give rise to inhibition of the covering tissues of the fetus with this antigen, and this could explain the positive results of detection of EPA-containing structures in that region (epidermis, membrane of the umbilical cord). The discovery of EPA in the blood vessels can be explained by the presence of this protein in the fetal blood serum.

The results of the study of cultural samples by the method of crossed immunoelectrophoresis followed by autoradiography revealed incorporation of the label only into the slow EPA peak (Fig. 2), although EPA was present in the fetal blood serum as both slow and fast peaks [1, 5]. It can be tentatively suggested that the fast peak, the synthesis of which has not yet been found in embryonic tissues, appears in the course of metabolic conversions of the slow peak, which become separated under the conditions of suspension culture. It may be that one particular tissue or organ is responsible for the synthesis of the past EPA peak.

The results of the study of EPA biosynthesis by the various embryonic tissues correlate with the frequent discovery of this protein in high concentrations in tumors of connective-tissue origin [6].

Immunofluorescence analysis of sections through the desmoid revealed the presence of EPA in tumor cells (Fig. 3a, b, c). However, EPA was found not only in tumors of connective-tissue origin, but also in tumor tissue of different genesis. Immunofluorescence analysis of sections through an adenocarcinoma of the ovary revealed EPA in the connective tissue; the tumor cells themselves, did not contain EPA (Fig. 3d, e, f).

There is thus reason to suppose that EPA is a product of the mesenchyme and that evidently it is actively produced during embryonic development. The synthesis of this protein is appreciably reduced at birth. However, its synthesis is resumed during neoplastic degeneration of tissues, especially in tumors of connective-tissue origin, where it is located in the tumor cells. It is an interesting fact that in tumors of non-connective-tissue origin the tumor cells did not contain this protein, although it was found in the underlying connective tissue. The phenomenon of embryonic reversion probably affects not only "true" tumor cells, but also the surrounding connective tissue.

LITERATURE CITED

1. V. V. Kalashnikov, S. A. Borisenko, and Yu. S. Tatarinov, Byull. Éksp. Biol. Med., No. 6, 725 (1976).
2. V. V. Kalashnikov, M. Yu. Vasil'ev, S. G. Voloshchuk, et al., Ontogenez, No. 2, 53 (1979).
3. Yu. S. Tatarinov, V. V. Kalashnikov, S. A. Borisenko, et al., Byull. Éksp. Biol. Med., No. 1, 50 (1975).
4. Yu. S. Tatarinov, D. M. Falaleeva, V. V. Kalashnikov, et al., Vopr. Med. Khim., No. 1, 88 (1977).
5. Yu. S. Tatarinov and V. V. Kalashnikov, Nature, 265, 638 (1977).
6. Yu. S. Tatarinov, V. V. Kalashnikov, M. Yu. Vasil'ev, et al., Lancet, 2, 1122 (1978).
7. I. L. Guesdon and S. Avrameas, Immunol. Meth., 11, 129 (1976).
8. J. Hamachima, J. G. Harter, and A. H. Coons, J. Cell Biol., 20, 271 (1964).
9. C. B. Laurell, Anal. Biochem., 10, 358 (1955).
10. G. Sainte-Marie, J. Histochem. Cytochem., 250 (1962).