IMMUNOFLUORESCENCE STUDY OF EMBRYONIC PREALBUMIN 1

LOCATION IN FETAL AND DEFINITIVE HUMAN TISSUES

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Embryonic prealbumin 1 (EPA-1), described by Kalashnikov et al. [4, 8], is an embryonic tumor-associated antigen, for it is a normal component of fetal blood serum and is found in homogenates of human malignant tumors [5, 7]. In native blood serum and in homogenates of adult human tumors EPA-1 as a rule cannot be detected by immunodiffusion methods [7]. EPA-1 is a glycoprotein containing about 2% of sulfate [6]. This antigen consists of two immunochemically identical electrophoretic fractions possessing the mobility of prealbumins [5]. It was shown previously that EPA-1 is synthesized in the mesenchyme and mesoderm of human fetuses at 6-12 and 20-24 weeks of development [1].

The object of this investigation was to study the location of EPA-1 in sections of fetal and definitive human tissues by an immunofluorescence method.

EXPERIMENTAL METHOD

Organs and tissues of human fetuses at 6-12 and 20-24 weeks of development, obtained from healthy pregnant women after medical abortions in Moscow maternity homes, and also organs and tissues of adults dying from accidental causes, obtained from forensic-medical mortuaries in Moscow were investigated. Antibodies against EPA-1 were isolated from monospecific antisera on immunosorbents by the method described previously [1]. The location of EPA-1 in fetal and definitive human tissues was studied by the indirect immunofluorescence method [10] on histological (5 μ) serial sections of material obtained after fixation in ethanol and acetic acid [11] and embedding in paraffin wax [14]. Isolated specific antibodies against EPA-1 were used as primary antibodies, and fluorescein isothiocyanate-labeled donkey antiserum against rabbit γ-globulin (from the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) as secondary antibodies. In control experiments the sections were treated with primary antibodies neutralized with a purified preparation of EPA-1 and with secondary antibodies, or with secondary antibodies alone.

EXPERIMENTAL RESULTS

Immunofluorescence study of sections of organs and tissues of human fetuses at 6-12 and 20-24 weeks of development showed that EPA-1 is present in most of the tissues studied (Table 1; Fig. 1); structures containing it, moreover, share a common origin — the embryonic mesoderm. These structures either arise directly from mesoderm (bone marrow cells, Wharton's jelly cells of the umbilical cord, epithelium of the renal tubules) or they are its derivatives — cells and fibers of mesenchyme (fibrous structures of the stroma and membranes of organs, perivascular zones, and mesenchymal cells proper).

The extensive spread of EPA-1 in the fetal connective tissue raised the question of whether this antigen is present also in definitive connective tissue. Immunofluorescence investigation of sections of 22 different organs and tissues of a healthy adult showed that EPA-1 is present in fibrous connective tissue, mainly in perivascular zones, in most of the organs studied (Table 2). The greatest intensity of fluorescence was found in sections of the kidney, spleen, and esophagus (Fig. 2). Besides the location of EPA-1 in sections of the

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TABLE 1. Results of Immunofluorescence Study of Location of EPA-1 in Organs and Tissues of Human Fetuses at 6-12 and 20-24 Weeks of Development

Organs and tissues studied	Fetuses at 6-12 weeks of development		Fetuses at 20-24 weeks of development	
	intensity of fluorescence	characteristics of fluorescent structure	intensity of fluorescence	characteristics of fluorescent structure
Umblicus	+++++	Cytoplasm of fibroblast-like cells of Wharton's jelly	++++	Connective-tissue fibers of ground substance
Skin-muscle flap	+++	Cytoplasm of mesenchymal cells proper and connective-tissue fibers of skin	++	Cytoplasm of fibroblast-like cells of dermis, connective-tissue fibers of perimysium of muscles
Bone	+++	Cytoplasm of cartilage cells	++	Connective-tissue fibers of periosteum
Kidney	+++	Connective-tissue fibers of stroma, cytoplasm of tubular epithelium	+	Connective-tissue fibers of stroma, basement membrane of tubular epithelium
Chorion	+++	Connective-tissue fibers of stroma membrane	N	N
Stomach	N	N	+	Connective-tissue fibers of tunica propria of mucous and serous membrane
Small intestine	++	Connective-tissue fibers of serous membrane	+	The same
Large intestine	N	N	+	
Lung	+	Fibrous structures of basement membrane of bronchi	+	Fibrous connective tissue of stroma
Heart	N	N	+	Fibrous connective tissue of perivascular zones
Liver	—	—	—	—
Brain	—	—	—	—

Legend. From + to +++++) intensity of fluorescence ranging from weak to very strong; —) fluorescence absent; N) not tested.

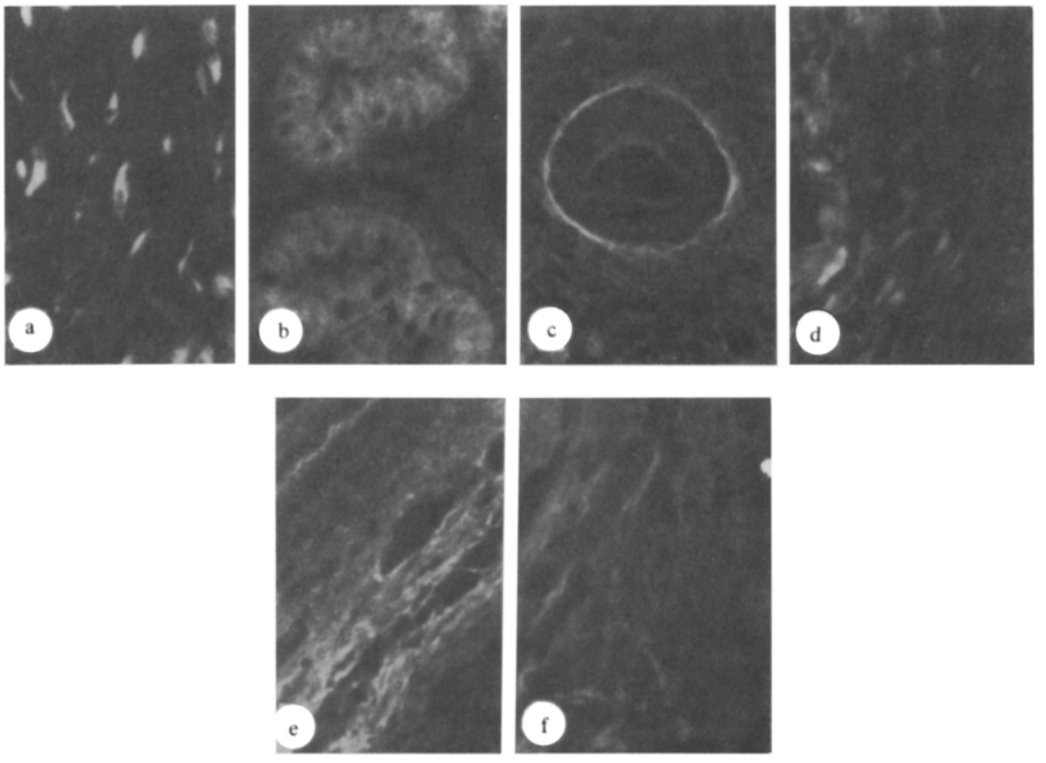

Fig. 1. Localization of EPA-1 in sections of organs and tissues of human fetus at 6-12 weeks of development. Sections through: a) umbilicus, b) kidney, c) lung, d) bone, e) skin, f) chorion. Here and in Fig. 2, immunofluorescence method; magnification: objective 40, ocular 5.

TABLE 2. Results of Immunofluorescence Study of Location of EPA-1 in Definitive Human Tissues

Organs and tissues studied	Intensity of fluorescence	Characteristics of fluorescent structure
Brain	–	–
Cerebellum	–	–
Skin	++	Fibrous connective tissue of perivascular zones
Muscle	+	The same
Costal cartilage	+	Ground substance of intercellular spaces
Heart	+	Fibrous connective tissue of perivascular zones
Lung	++	The same
Tongue	+	
Esophagus	+++	
Stomach	+	
Small intestine	+	
Large intestine	+	
Spleen	+++	
Liver	+	
Pancreas	+	
Kidney	+++	Fibrous connective tissue of perivascular zones and stroma of organ
Adrenal	++	The same
Testis	+	
Ovary	++	
Uninary bladder	+	Fibrous connective tissue of perivascular zones
Prostate	+	The same
Uterus	+	

Legend. –) Absence of specific fluorescence in sections; + to +++) intensity of fluorescence ranging from weak to average.

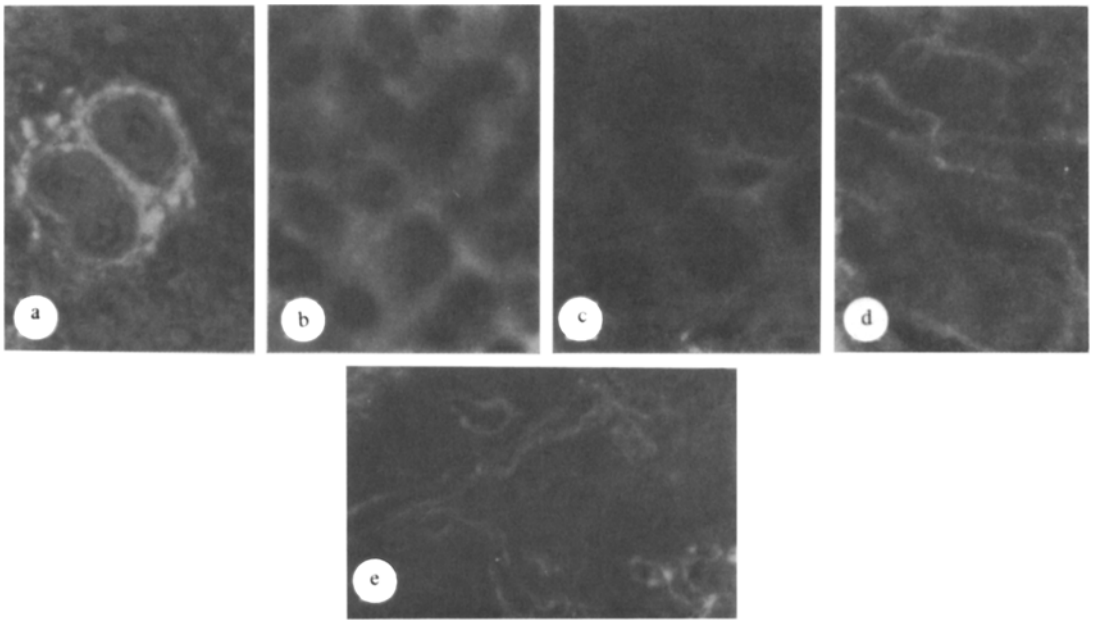

Fig. 2. Localization of EPA-1 in sections of organs and tissues of healthy adult. Sections through: a) spleen, b) costal cartilage, c) kidney, d) adrenal, e) ovary.

kidney, adrenal, testis, and ovary, described above, specific fluorescence also was found in fibrous structures of the connective-tissue stroma of these organs. EPA-1 also was present in the ground substance of the cartilage (Fig. 2).

By means of the standard test system EPA-1 was identified in a concentrated glycoprotein fraction from a mixture of blood sera from healthy adult blood donors, obtained by the method described in [5], in a quantity corresponding to 25 ng EPA-1/ml native serum. This "normal" serum level of EPA-1 is comparable with that of certain embryonic tumor-associated antigens (alpha-fetoprotein — up to 20 ng/ml [13], carcinoembryonic antigen — up to 12.5 ng/ml [12]).

Comparison of the results on EPA-1 biosynthesis in fetal tissues and its immunofluorescence localization in them led to the conclusion that EPA-1 in the human fetus is a product of cells of the mesoderm and its derivative, mesenchyme [1]. The discovery of this antigen in normal adult blood serum and also the presence of EPA-1 in connective-tissue structures of various definitive organs suggests that this antigen is produced in the adult individual also, evidently by connective-tissue cells. To sum up all that has been stated above, it was concluded that EPA-1 is a product of both fetal and definitive connective tissue. This conclusion is also confirmed by the ability of fetal and definitive human fibroblasts to produce EPA-1 *in vitro* [2, 9].

EPA-1 has been found by an immunodiffusion method, capable of detecting only antigens extractable by salt solutions, in homogenates of fetal [3] and malignant tumors [7], but not in the corresponding definitive tissues [7]. Meanwhile, by means of an immunofluorescence method, capable of detecting antigens insoluble in salt solutions also, we found EPA-1 not only in fetal, but also in definitive human tissues. The disagreement between the results of immunodiffusion and immunofluorescence methods of detection of EPA-1 can be explained on the assumption that there are two forms of EPA-1: a saline-soluble form of fetal type, produced by embryonic and tumor tissues and detectable by various immunodiffusion methods, and a saline-insoluble form of definitive type, produced by adult human connective-tissue cells, and detectable only by the immunofluorescence method.

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