Localisation of embryonic prealbumin in formalin-fixed human fetal and adult tissue

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SUMMARY The presence of embryonic prealbumin (EPA) has been confirmed in fetal fibroblasts, chondrocytes, and distal tubular epithelial cells by an indirect immunoperoxidase technique. EPA has often been found also in the stromal cells of benign and malignant mesodermal tumours, but not in the epithelial cells of benign and malignant epithelial tumours. That EPA is not an exclusive product of neoplastic mesodermal cells is demonstrated by our finding of EPA in fibroblasts of granulation tissue, irradiated fibroblasts, and in distal tubular epithelial cells of miscellaneous adult kidneys.

Human embryonic prealbumin (EPA) is a recently described fetal protein which has been shown to be non-identical with a variety of oncofetal antigens, including alphafetoprotein, carcinoembryonic antigen, pregnancy-specific glycoprotein and beta-oncofetal antigen.¹ It is a glycoprotein containing 21% carbohydrate and there are two immunologically identical forms of the protein, one having a molecular weight of 41 000, the other 68 000. With an immunodiffusion technique, EPA is detectable in normal fetal serum at an early stage of development, the serum concentration rising steadily and reaching a peak between the 20th and 30th week of gestation. Immunodiffusion studies have also demonstrated that it is present in homogenates of tumours, especially those of connective tissue origin, and in over 17 different fetal adult fibroblast cell lines.²⁻⁴ With the fluorescent antibody technique Kalashnikov and his colleagues³ have shown it can be localised within placenta, umbilical cord, fetal tissues (including skin, bone, and kidney) and in the stroma surrounding some epithelial tumours. In our study, using an indirect immunoperoxidase technique, we have examined formalin-fixed paraffin embedded human fetal and adult tissues for the presence and distribution of EPA, including benign and malignant tumours. In addition, to determine whether or not EPA is truly an oncofetal protein, tissues and organs from patients with various non-neoplastic conditions were studied.

Material and methods

Paraffin-embedded blocks of human tissues which had been fixed in either formol saline or neutral buffered formalin and stored for up to four years, were obtained from the files of the pathology departments in Aberdeen and Glasgow. The EPA antiserum (provided by Professor Tatarinov) was raised in rabbits using semi-purified EPA preparations and rendered monospecific by subsequent absorption using pooled human plasma and extract of normal tissues.

IMMUNOPEROXIDASE TECHNIQUE

The immunoperoxidase techniques used in both departments were essentially the same except that the addition of trypsin before the addition of the normal swine serum was not carried out by the Glasgow department. Despite this there did not appear to be any qualitative difference in the preparations from the two departments.

Sections (5 μ m) were made from paraffin-embedded blocks of tissue. After taking the sections to water endogenous peroxidase was blocked by treating the sections with 0.5% H₂O₂ in methanol for 30 min⁵ and the sections were then washed in distilled water. They were treated with 0.1% trypsin in 0.1% CaCl₂ (pH 7.8) for 20 min at 37°C.⁶ In the Aberdeen laboratory trypsinisation increased the intensity of the positive staining.

Non-specific background staining was reduced by exposing sections to 1/5 dilution of normal swine

serum. The specific EPA antiserum was used at a dilution of 1/200 (30 min). Swine antirabbit immunoglobulin (Dakopatts A/S) was then applied (dilution 1/40) followed by a 1/100 dilution of the peroxidase-antiperoxidase (PAP) complex (Dakopatts A/S). Peroxidase activity was demonstrated using the diaminobenzidine reaction.

Each test section was compared with an absorption control. In most cases, owing to the shortage of purified EPA (provided by Tatarinov), fetal serum was used for this purpose. Sections were read independently by two observers and the degree of staining was graded using an arbitrary scale: ++ strong, + moderate, ± weak.

Results

The distribution of EPA in miscellaneous benign and malignant tumours is shown in Tables 1 and 2. EPA is detectable in 34% of benign tumours and in 41% of malignant tumours, although a much higher percentage of malignant connective tissue tumours (67%) stain positively for EPA than do benign connective tissue tumours (33%). In most tumours EPA is present only in cells of mesodermal origin, especially fibroblasts (Fig. 1). EPA is not detectable in epithelial cells of benign or malignant tumours. Endothelial cells within the tumours also contain EPA and this is well illustrated by the striking perivascular deposition of EPA in glioblastoma multiforme (Fig. 2).

Screening of fetal tissues shows that EPA is detectable in the mesenchymal cells of umbilical cord, fibroblasts especially of the upper dermis, cartilage and in the distal renal tubular epithelium (Fig. 3). "Normal" adult (male and female) tissues

Table 1 Incidence of embryonic prealbumin (EPA) in 61 benign tumours

Benign tumours	Proportion of EPA-positive tumours	%
<i>Connective tissue</i>		
Dermatofibroma (sclerosing angioma)	5/9	56
Fibroma/thecoma (ovarian)	2/13	15
Leiomyoma (uterine)	1/5	20
Haemangioma (capillary/cavernous)	2/5	40
<i>Epithelial</i>		
Papillary serous cystadenoma of ovary	6/15	40
Mucinous cystadenoma of ovary	1/8	12.5
<i>Neural</i>		
Miscellaneous brain tumours: astrocytoma × 2; oligodendroglioma; meningioma	2/4	50
<i>Mixed</i>		
Pleomorphic salivary adenoma	2/2	100
Total	21/61	34

Table 2 Incidence of embryonic prealbumin (EPA) in 81 malignant tumours

Malignant tumours	Proportion of EPA-positive tumours	%
<i>Connective tissue</i>		
Sarcoma		
Fibrosarcoma	0/2	—
Leiomyosarcoma	7/10	70
Unclassified	3/5	60
Miscellaneous (osteosarcoma × 2; liposarcoma chondrosarcoma × 2; synovial sarcoma; rhabdomyosarcoma)	5/7	71
<i>Epithelial</i>		
Papillary serous cystadenocarcinoma of ovary	5/12	42
Mucinous cystadenocarcinoma of ovary	4/9	44
Carcinoma of ovary (including metastatic carcinoma)	3/6	50
Carcinoma of breast (anaplastic ± scirrhous reaction)	0/10	—
Miscellaneous malignant epithelial tumours (including bladder, bronchus, bowel, and prostate)	1/12	8
<i>Neural</i>		
Glioblastoma multiforme	2/2	100
<i>Mixed</i>		
Testicular teratoma	4/6	66
Total	34/81	41

Fig. 1 High power view of a dermatofibroma showing positive staining for EPA within cytoplasm of fibroblasts (arrowed). Immunoperoxidase/methyl green × 650.

and organs including brain, pituitary, thyroid, salivary gland, heart, liver, kidney, adrenal, small and

Fig. 2 Strongly positive staining for EPA in endothelial cells and around vessel walls in a glioblastoma multiforme. Immunoperoxidase methyl green $\times 256$.

Fig. 3 High power view of strongly positive granular staining for EPA in distal tubular epithelial cells of fetal kidney. Immunoperoxidase methyl green $\times 650$.

large bowel, spleen, bladder, cartilage, and skeletal muscle are all negative for EPA. Apart from the weak staining for EPA in periosteum the only significant positive staining was seen in lung alveolar cells and endothelial cells of the interstitium of the testis. However, endothelial cells in other organs did not stain positively for EPA.

That EPA is not simply a product of neoplastic cells is shown by its frequent presence in a variety of non-neoplastic conditions (Table 3). This also shows the precise distribution of EPA in such tissues. EPA is frequently detectable in adult kidneys (Table 3). Apart from its presence in distal tubular epithelial cells periglomerular deposition of EPA is a notable feature in some adult kidneys (Fig. 4).

Discussion

Our findings concerning the distribution of EPA agree with those of Kalashnikov and his colleagues.³ As expected EPA appears to be a product of fetal mesenchymal cells, especially the fibroblast and chondrocyte. A particularly striking feature, however, is the presence of strongly positive staining of fetal distal tubular epithelial cells. This is also a feature of some adult kidneys. It may represent renal excretion of EPA with subsequent reabsorption.

Our detection of EPA in a high proportion of adult mesodermal tumours is in agreement with earlier work in which it has been shown, with a simple double immunodiffusion technique, that EPA is present in various mesodermal tumour homogenates.^{4,7} The frequency of detection of EPA in the malignant mesodermal tumours in our series is 67%, while in a recent study by Tatarinov⁷ the incidence of EPA in malignant mesodermal tumours is 75%. However, our findings do not accord with those of Tatarinov⁴ in breast cancer, where he was able to detect EPA in 48% of homogenates of breast

Fig. 4 High power view of crescentic deposition of EPA in a glomerulus (adult kidney—chronic pyelonephritis). Immunoperoxidase/methyl green $\times 650$.

cancers, whereas in 10 such cancers we did not find any which stained positively for EPA; indeed the vast majority of all malignant epithelial tumours studied do not appear to produce EPA. At present there is no obvious explanation for the difference between Tatarinov's findings⁴ in respect of breast cancer and our own.

The most important aspect of our study was the detection of EPA in a variety of non-neoplastic tissues. While it is perhaps not surprising to find EPA in fibroblasts in granulation tissue, which are rapidly dividing cells of mesodermal origin, and also in irradiated fibroblasts, we did not anticipate finding EPA in renal tubular epithelial cells in patients who did not have tumours. This is thought to be a result of reabsorption rather than synthesis in the kidney itself. The apparent high frequency of occurrence suggests that the appearance of EPA in the circula-

Table 3 Incidence of embryonic prealbumin (EPA) in 38 non-neoplastic conditions

	Proportion of EPA-positive non-neoplastic specimens	%	Comment
Granulation tissue	8/10	80	EPA present in fibroblasts
Fibromatosis (palmar and plantar)	3/5	60	EPA present in fibroblasts
Radiation (skin)	7/8	88	Dermal fibroblasts (both normal and abnormal) contain EPA
Renal conditions			
miscellaneous (pyelonephritis; renal cysts; senile nephrosclerosis; pyonephrosis; hydronephrosis; amyloid)	4/6	66	Periglomerular deposition of EPA usually in association with glomerular sclerosis.
transplant rejection $\times 4$	4/4	100	EPA also noted in droplets within distal tubular epithelial cells and in tubular lumina
Amyloidosis (secondary type) (liver, thyroid, rectum, tonsil)	3/5	60	Except in the case of the amyloid goitre there is no deposition of EPA in relation to amyloid
			EPA is demonstrable in connective tissue of liver and in subepithelial zone of rectum
Total	29/38	78	

tion can be induced by a variety of stimuli. One possibility consistent with this is that EPA may be an acute-phase reactant which, although absent from "normal" adult sera, becomes detectable in serum following various stimuli, such as surgical trauma and infection. An example of such an acute-phase reactant is C-reactive protein. Whether or not EPA is a true acute-phase reactant can be shown following development of a sensitive immunoassay. Work to establish such an assay is now in progress. Finally, it remains to be seen whether EPA is a clinically valuable serum marker for malignant mesodermal tumours.

References

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