IMMUNOCHEMICAL STUDY OF ANDROGEN-BINDING HUMAN β-GLOBULIN IN NORMAL SUBJECTS AND CANCER PATIENTS

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The discovery of α-fetoprotein in hepatocarcinoma [1, 6] stimulated the search for and study of oncofetal antigens [7, 9-11]. Considering the important role of steroid hormones in cell differentiation processes, and on the basis of investigations [3, 5] which have demonstrated hormonal disturbances during growth of tumors, significant changes can be anticipated in proteins binding steroid hormones during carcinogenesis.

The object of this investigation was an immunochemical study of androgen-binding β-globulin, which is absent from healthy human serum, in some forms of malignant tumors.

EXPERIMENTAL METHOD

Antiserum against androgen-binding protein (ABP) was obtained by immunizing rabbits by the usual scheme with a preparation isolated by affinity chromatography from an extract of human abortion material on a specific sorbent containing immobilized testosterone. Testosterone was immobilized as follows: sepharose CL-4B (from Pharmacia Fine Chemicals) was bound with heptamethylenediamine by the periodate oxidation method, and then with testosterone-C-17-hemisuccinyl by the carbodi-imide method. The resulting antiserum was specifically absorbed with healthy human plasma (from blood donors) and a monospecific test system was then formed with the preparation used for immunization.

The immunochemical study of ABP was carried out by titration with the standard test system in Khramkova and Abelev's immunodiffusion technique [8] and also by crossed immunoelectrophoresis in agarose gel [12]. Tissue extracts were prepared in Tris-glycine buffer, pH 8.3, with the addition of 0.1% of the detergents Triton X-100 and Tween-80, by freezing and thawing 3 times followed by centrifugation at 8000 rpm for 30 min.

EXPERIMENTAL RESULTS

The distribution of ABP in normal human tissue extracts and biological fluids was studied by immunodiffusion analysis. The antigen was found in the highest titers in extracts of the prostate gland (1:8-1:32) and sperm (1:4-1:8), and also in low concentration in extracts of human spleen and adult human lung. ABP also was detected in neonatal blood serum, in retroplacental blood serum, and placental extracts.

The relative mobility of the ABP was determined by immunoelectrophoretic analysis; its value was 0.50 ± 0.02, so that this antigen could be classed as a β-globulin [2] (Fig. 1).

The study of extracts of malignant tissues (Fig. 2) shows that ABP was present in tissue extracts from malignant tumors of the breast, kidney, stomach, large intestine, brain, and ovary. The results of immunodiffusion analysis of androgen-binding β-globulin in tissue extracts of tumors are given in Table 1. They show that this protein participates in the "antigenic divergence phenomenon" [4], which was expressed as the appearance of antigens not typical of the normal original tissue, in tumor tissue.

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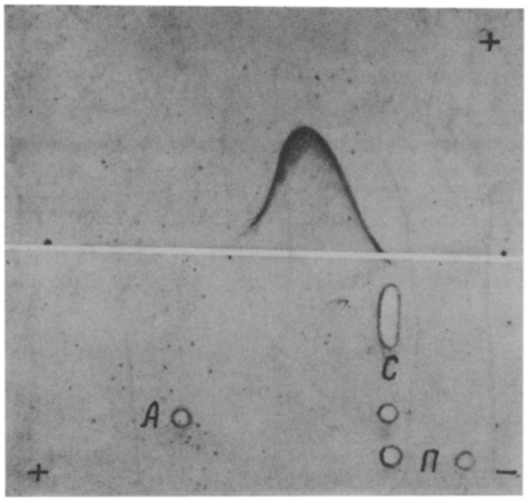

Fig. 1

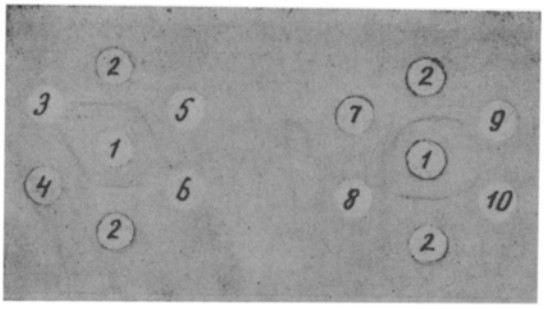

Fig. 2

Fig. 1. Immunoelectrophoretic analysis of ABP in adult human prostate extract. Electrophoresis in first direction in 1% agarose gel in 0.5 M veronal-medinal buffer, pH 8.6, at 10 V/cm for 40 min; electrophoresis in second direction in the same gel containing 2.5% antiserum (by volume) at 2 V/cm for 15 h. Volume of sample 20 μl. A) Albumin zone, P) pyronine zone; S) start. Stained with 1% aqueous solution of Coomassie R250.

Fig. 2. Immunodiffusion analysis of ABP in blood sera and organ and tissue extracts from healthy subjects and cancer patients. 1) Antiserum against ABP, 2) preparation of androgen-binding β-globulin, 3) blood serum of healthy donor, 4) extract of Wilms' tumor, 5) blood serum of patient with Wilms' tumor, 6) extract of adult human kidney, 7) extract of brain glioblastoma, 8) healthy human brain extract, 9) extract from adenocarcinoma of stomach, 10) blood serum from patient with adenocarcinoma of stomach.

TABLE 1. Immunodiffusion Analysis of Androgen-Binding β-Globulin in Tumor Tissue Extracts

Material studied (malignant tissue)	Number of specimen		ABP titer
	total	positive	
Stomach	5	4	1:1—1:8
Kidney	5	4	1:2—1:64
Lung	7	5	1:2—1:4
Ovary	6	5	1:2—1:32
Breast	12	3	1:1—1:2
Brain	24	14	1:1—1:8
Pleura	2	2	1:1—1:4
Uterus	3	—	—
Chorion	9	9	1:4—1:32

The study of blood sera from cancer patients in order to detect ABP revealed this antigen in about 50% of samples of blood serum from patients with various forms of malignant tumors with a histologically confirmed diagnosis. These results are given in Table 2.

As Table 2 shows, this antigen may be secreted into the blood in certain types of cancer and, consequently, it is a tumor-associated antigen.

In crossed reactions this antigen was found to be not identical with antigens possessing affinity for steroid hormones, namely: α-fetoprotein, trophoblast-specific β-glycoprotein, and estrogen-binding α-globulin, and also with antigens of placenta and amniotic fluid studied previously in the writers' laboratory [13].

The appearance of androgen-binding β-globulin in malignant tissues and in the blood serum of cancer patients may indicate shifts in adrogen metabolism during neoplastic growth.

TABLE 2. Immunodiffusion Analysis of Androgen-Binding β-Globulin in Blood Serum of Cancer Patients

Localization of tumor	Number of specimens of serum		Titer of ABP
	total	positive	
Lungs	36	22	1:1—1:2
Stomach	11	4	1:2—1:8
Rectum	16	8	1:1—1:2
Kidney	15	9	1:2—1:8
Skin	3	—	—
Pleura	3	2	1:1—1:2
Female reproductive organs	7	—	—
Ovary	3	—	—
Control (blood serum from healthy donors)	35	—	—

The possible participation of ABP in hormonal regulation of metabolism in man may also be postulated. Conclusions regarding the concrete biological function of androgen-binding β-globulin in man under normal conditions and during neoplastic growth can be drawn only after its isolation in a pure form and further study.

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