ONCOLOGY

Detection of Phosphotyrosine-Containing Proteins in the Nuclear Matrix of Some Tumors

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Tyrosine-containing proteins were detected by the immunoperoxidase method in the nuclear matrix of the liver and some tumors of mice. Two strips with molecular weights of about 180 and 170 kD are characteristic of hepatoma 22a and Ehrlich's ascitic carcinoma. Immunoelectron study with colloid gold showed that tyrosine-containing proteins and fibronectin are commonly present in the nucleus and cytoplasm.

Key Words: *nuclear matrix; phosphotyrosine; tumors*

Our previous studies showed the predominance of high-molecular proteins in the nuclear matrix of tumors in comparison with normal tissues [1,2]. A microtubule-associated protein, MAP2-like protein p260, and fibronectin, which is not found in the nuclei of normal cells, were detected in this group of proteins in hepatomas [4].

In addition, numerous phosphoproteins, among them two strips with molecular weights of about 180 and 170 kD resistant to alkaline hydrolysis, were found in the nuclear matrix of rat hepatoma 27. This indicated the probable presence of phosphotyrosine residue in them [9] (Fig. 1).

With this in mind, and considering the importance of tyrosine phosphorylation for proliferation and tumor growth, we investigated the nuclear matrix of hepatoma 22a and Ehrlich's carcinoma in comparison with normal mouse liver by the immunochemical reaction with peroxidase-conjugated antibodies. Antibodies conjugated with colloid gold were used to detect the ultrastructural localization of these proteins. In addition, the localization of fibronectin in hepatoma 22a cell nuclei was identified.

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MATERIALS AND METHODS

Cell nuclei and nuclear matrix were isolated from mouse liver as described previously [6]. Ehrlich's ascitic carcinoma and hepatoma 22a cells transplanted to (CBA×C57Bl/6) F_1 mice were destroyed by osmotic shock in distilled water and the nuclei and nuclear matrix were isolated from them as described previously [4].

Protein phosphorylation in isolated nuclei (for electrophoresis) or cells (for electron microscopy) was carried out in 10 mM Tris-HCl buffer, pH 7.5, for 20 min at 30°C [3].

The proteins of nuclei and nuclear matrix were separated by polyacrylamide gel electrophoresis in the presence of sodium lauryl sulfate (SDS) [7]. Some of the electrophoregrams were stained with Coomassie blue. Proteins from other electrophoregrams were transferred onto nitrocellulose filters by electroblotting [8], and strips of proteins containing phosphotyrosine residue were detected using monoclonal antibodies to phosphotyrosine-containing proteins and antibodies to peroxidase-conjugated murine IgG (Dia-M, Moscow). The transfer of proteins onto the nitrocellulose filters was monitored by staining with 0.1% amido black.

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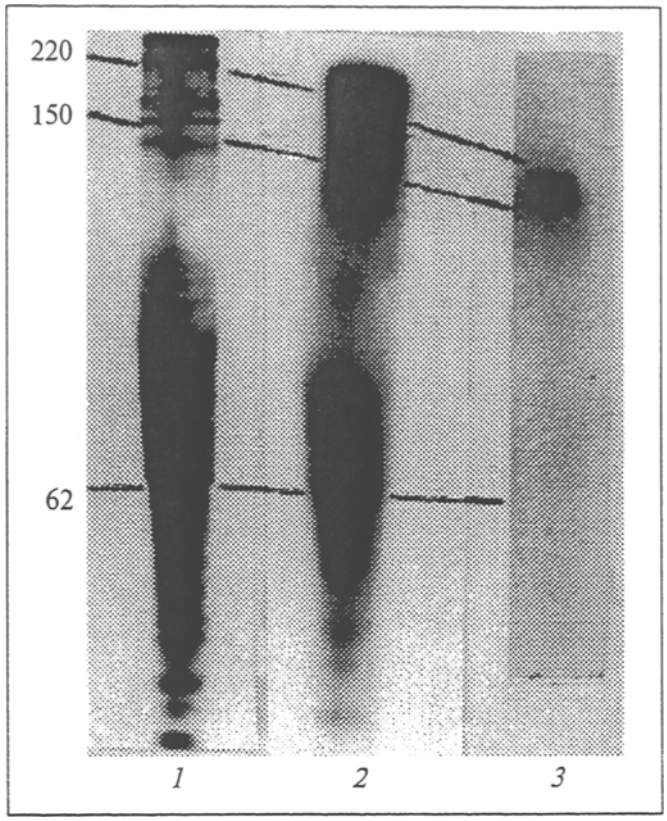

Fig. 1. Detection of phosphoproteins resistant to alkaline hydrolysis in the nuclear matrix of solid hepatoma 27 in rats. *1*) electrophoregram in 7.5% SDS–polyacrylamide gel stained with Coomassie blue; *2*) autoradiogram of a similar gel; *3*) autoradiogram of the same gel after 2-hour treatment with 1 N NaOH at 40°C.

For electron microscopic localization of tyrosine-containing proteins and fibronectin whole cells of ascitic tumors were treated with the respective antibodies (Dia-M) and antimurine antibodies conjugated with colloid gold (10 nm particles, Sigma).

RESULTS

The immunocytochemical test with peroxidase-conjugated antibodies clearly points to the presence of phosphotyrosine-containing proteins in the nuclei and nuclear matrix of both normal liver and tumors. On electrophoregrams they are seen predominantly in the high-molecular region. Bands corresponding to molecular weights of 160 and 140 kD are seen on the normal liver strip. In the nuclear matrix of hepatoma 22a and Ehrlich's ascitic carcinoma, bands with molecular weights of about 180 and 170 kD are prominent, in addition to the above bands (Fig. 2).

These sharply expressed bands correspond to the position of phosphorylated proteins with phosphorus label resistant to alkaline hydrolysis, which were detected in rat hepatoma 27 preparations (Fig. 1), thus confirming that these proteins do indeed contain phosphotyrosine residues.

The presence of similar fractions of phosphotyrosine-containing proteins in the nuclear matrix of two hepatomas, solid rat and ascitic murine, and of Ehrlich's ascitic carcinoma suggests that high-molecular phosphotyrosine-containing proteins corresponding to these two bands are characteristic of the nuclear matrix of malignant tumors and are evidently related to tumor growth. Their biological role can only be hypothesized.

Immunoelectron-microscopic study revealed a reaction to phosphotyrosine residues in both the nucleus and cytoplasm of all tumors studied, thus indicating that this type of protein is not confined to any specific localization (Fig. 3, *a*).

Previously we detected fibronectin in isolated hepatoma cell nuclei [4]. However, since it was found at the periphery of the isolated nuclei and nuclear matrix, the possibility of its adsorption in the course of isolating these structures could not be ruled out.

A study of intact cells carried out during this investigation revealed fibronectin in both the nuclei and cytoplasm of ascitic tumor cells (Fig. 3, *b*), thus confirming its presence in the nuclear matrix of malignant tumors.

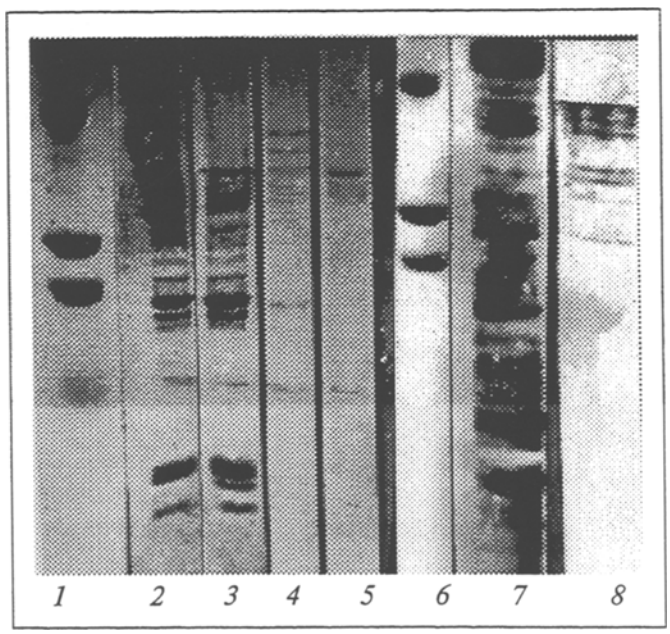

Fig. 2. Electrophoregram of proteins in 7.5% polyacrylamide gel in the presence of SDS. *1*, *6*) molecular weight standards (myosin, 200 kD; β-galactosidase, 116 kD; phosphorylase, 98 kD). *2*, *3*, *7*) Coomassie blue staining: *2*) hepatoma 22a; *3*) normal liver; *7*) Ehrlich's ascitic carcinoma. *4*, *5*, *8*) immunoperoxidase staining for phosphotyrosine residue: *4*) hepatoma 22a; *5*) normal liver; *8*) Ehrlich's ascitic carcinoma.

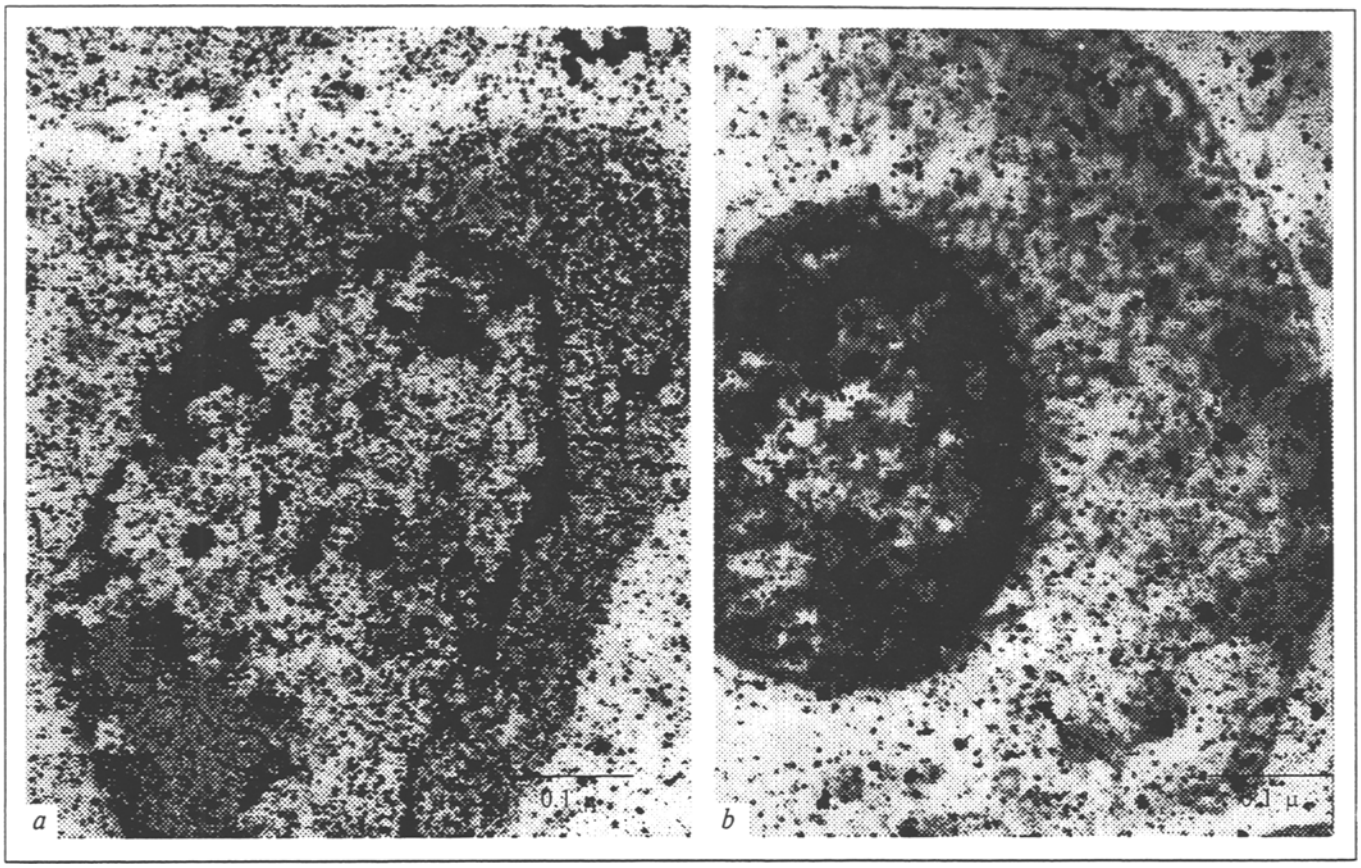

Fig. 3. Immunoelectron detection of phosphotyrosine–containing proteins (*a*) and fibronectin (*b*) in slices of ascitic hepatoma 22a cells. Uranyl acetate staining. ×16,000.

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