2. V. I. Dontsov and T. A. Ismailov, *Pat. Fiziol.*, № 5, 65-66 (1984).
3. A. A. Podkolzin and E. P. Markin, *Izvestiya Akad. Nauk SSSR*, **54**, № 10, 40 (1990).
4. E. N. Chirkov and Yu. N. Babaev, *Electromagnetic Nature of Immunity and Cell Differentiation* [in Russian], Akad. Naul SSSR, Inst. Kibernetiki, № 62, 1-18 (1987).
5. A. J. Cunningham, *Nature*, **207**, 1106-1107 (1965).
6. E. Ettlenne, P. Hoeng, and R. Frankel, *Effects of Magnetic Fields on Ca Transport in Isolated Muscle Microsomes: NSWC/URSI Spring Meet. Seatle*, Washington (1979), p. 442.
7. J. Walleczek and R. P. Liburdi, *FEBS Lett.*, **271**, № 1-2, 157-160 (1990).

Immunochemical Identification of Some High-Molecular Proteins in Tumor Nuclear Matrix

L. S. Filatova and I. B. Zbarskii

UDC 616-006-018.1-008.939.6-078.33

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 10, pp. 418-421, October 1993
Original article submitted April 26, 1993

Key Words: *nuclear matrix; fibronectin; hepatomas*

Extraction of isolated cell nuclei with a detergent, 0.14 M NaCl, and 2 M NaCl results in an insuluble residue consisting mainly of nonhistone proteins and retaining the shape and morphological structure of the nucleus. This residue is usually reffered to as the "nuclear matrix" (NM) or "nuclear skeleton."

The NM is not only the structural basis of the nucleus but is also involved in important biological functions, being the site of DNA replication and transcription and of mRNA transport to the nuclear envelope [3].

The NM of tumor cells is characterized by a predominance of the high-molecular protein group [1]. These proteins are virtually unidentified. The major components of this group are pore complex glycoproteins [6], DNA topoisomerases [8], and the MAP-2-like protein p260 [11].

A recent communication from Sauermann's laboratory reports the detection in HeLa cell nucler matrix of fibronectin, a normal component of extracellular matrix heretofore undetected in the nucleus [13]. Fibronectin has also been found in cell nuclei of some hepatomas [5].

N. K. Kol'tsov Institute of Developmental Biology, Russian Academy of Sciences, Moscow

The findings mentioned above are limited to the immunochemical reaction to fibronectin and do not relate to its fine localization in nuclear structures.

We studied NM isolated from a solid rat hepatoma 27 and ascites mouse hepatoma 22a and compared it to normal rat liver.

MATERIALS AND METHODS

Nuclei from the liver and hepatoma 27 were isolated according to Blobel and Van Potter's method [4] in a modification used in our laboratory [9]. Hepatoma 22a cells transplanted in F_1(CBA×C57Bl$_6$) mice were destroyed by incubation in distilled water and treatment in a Potter-Elvehjem homogenizer. The nuclei were then sedimented at 800 and 20,000 g in 1.8 M sucrose. The NM was prepared by extraction of isolated nuclei with 0.5% Triton X-100, treatment with 100 μg DNase I and RNase each in 5 ml of 20 mM Tris-HCl buffer, pH 7.4, containing 0.2 M sucrose, 2 mM $MgCl_2$, and 3 mM $CaCl_2$. Then the nuclear sap and chromatin were successively extracted with 0.14 M and 2 M NaCl [9].

Preparations of NM were then subjected to electrophoresis in polyacrylamide gel [10]; the pro-

0007-4888/93/0010-1281$12.50

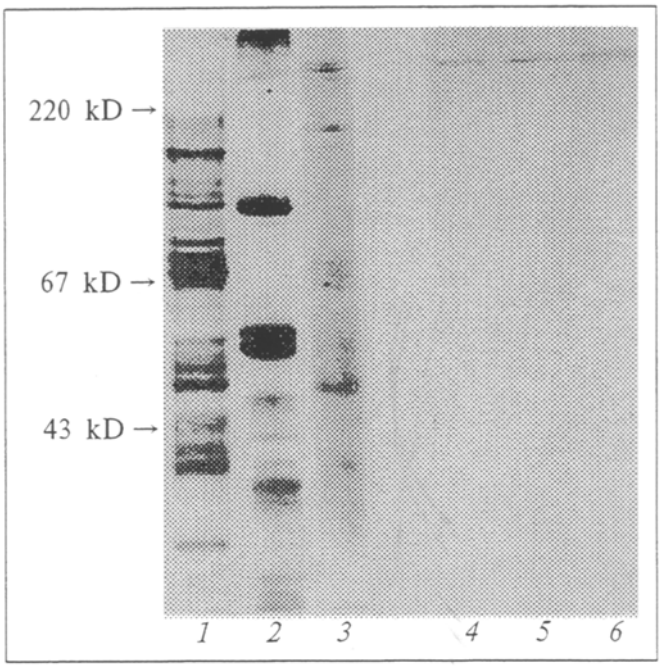

Fig. 1. Immunochemical identification of p260 protein in nuclear matrix. *1–3*) Coomassie blue staining; *4–6*) immunoperoxidase staining with monoclonal antibody to p260; *1,4*) normal rat liver; *2,5*) mouse ascites hepatoma 22a; *3,6*) rat solid hepatoma 27. At left: molecular weight standards.

teins were transferred to nitrocellulose filters [12] and protein p260 and fibronectin were detected by immunoblotting with monoclonal antibodies to mouse IgG conjugated with peroxidase. In the control the proteins were stained with 0.1% amido black.

An indirect immunological reaction with antibodies to mouse immunoglobulin conjugated with colloid gold was used for electron microscopic localization of the proteins studied.

Monoclonal antibodies to MAP-2-like protein p260 (microtubule associated protein 2) were graciously provided by Dr. Hiroshi Nakayasu (Kyoto, Japan), and antibodies to fibronectin by Dr. O. Yu. Printseva from the Institute of Experimental Cardiology of the Russian Academy of Medical Sciences.

For electron microscopy isolated nuclei and nuclear matrices were fixed in 2.5% glutaraldehyde and the immune reaction was performed [7]. The material was then processed with alcohols and acetone and embedded in epon-araldite. Ultrathin sections were examined under a JEM-7A electron microscope (Japan).

RESULTS

Coomassie blue staining of normal hepatocytes as well as electrophoregrams of hepatoma cells revealed a few dozen protein bands. In tumor preparations high-molecular bands predominated. By immunoblotting with antibody to p260 only one band corresponding to a molecular weight of 260 kD was revealed in electrophoregrams of nuclei and nuclear matrices of both normal liver and hepatomas (Fig. 1).

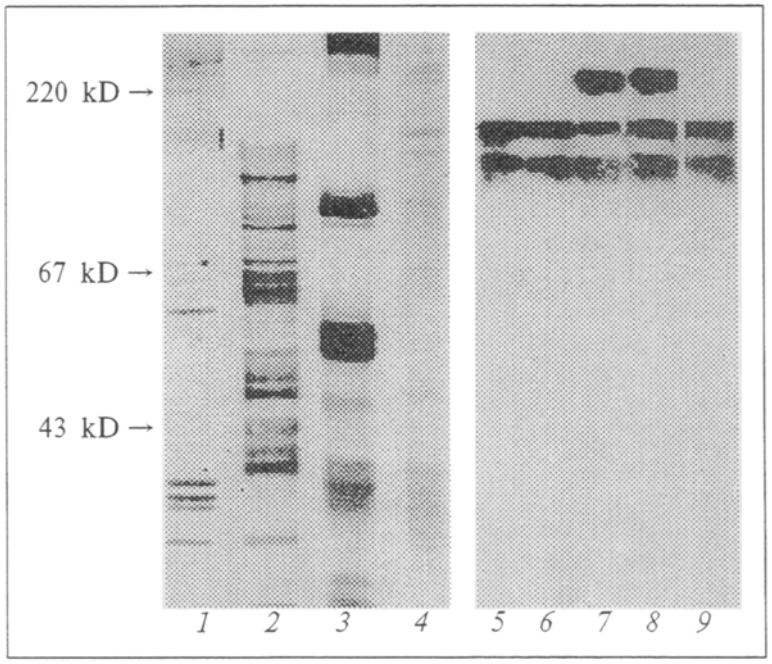

Fig. 2. Immunochemical identification of fibronectin. *1–4*) Coomassie blue staining; *5–9*) immunoperoxidase staining with monoclonal antibody to fibronectin; *1,5*) normal rat hepatocyte nuclei; *2,6*) normal rat liver nuclear matrix; *3,7*) mouse ascites hepatoma nuclear matrix; *4,8*) rat solid hepatoma nuclear matrix; *9*) bovine serum albumin (specificity control). A specific 250 kD bamd is seen only in tumor nuclear matrix. Bands around 150 and 180 kD are nonspecific.

A high-molecular band corresponding to fibronectin was detected in the test with antibody to this protein only in preparations from tumors but not normal liver nuclei or NM. Two more bands of lower molecular weight stained in this test. As these bands were revealed in all preparations, as well as in the control free of direct antigen, they were certainly nonspecific (Fig. 2).

In the electron microscopic study protein p260 is detected as dots uniformly distributed over the nuclei and NM, mainly inside them (Fig. 3, *a*). Fibronectin is detected only in tumor preparations at the periphery of the nuclei and is practically absent from the intranuclear area (Fig. 3, *b*).

Our results show that besides the known components (DNA-topoisomerase II, pore complex glycoproteins, and DNA and RNA polymerases) both proteins, p260 and fibronectin, are significant constituents of the nuclear matrix. The presence of fibronectin only in tumor preparations may be partly responsible for the predominance of

this group of proteins in tumor NM. Hence, our results confirm the presence of fibronectin in nuclei and NM of experimental malignant tumors [5] and HeLa cells [13].

However, no data on the fine localization of fibronectin in tumor nuclei are to be found in the literature. The discovery of this protein at the nuclear periphery may indicate its penetration from the extracellular matrix through the nuclear envelope.

The ability of hepatocytes to restore and maintain their organotypical structure *in vitro* and to inhibit the production of α-fetoprotein during cultivation in collagen gel or cocultivation with a nonparenchymal epitheliocyte strain of hepatic origin [2] may be relevant to our results. In other words, it may indicate that proteins of the extracellular matrix may influence cell proliferation and transformation. The appearance of fibronectin and, possibly, other proteins of the extracellular matrix may be associated with their effects of differentiation.

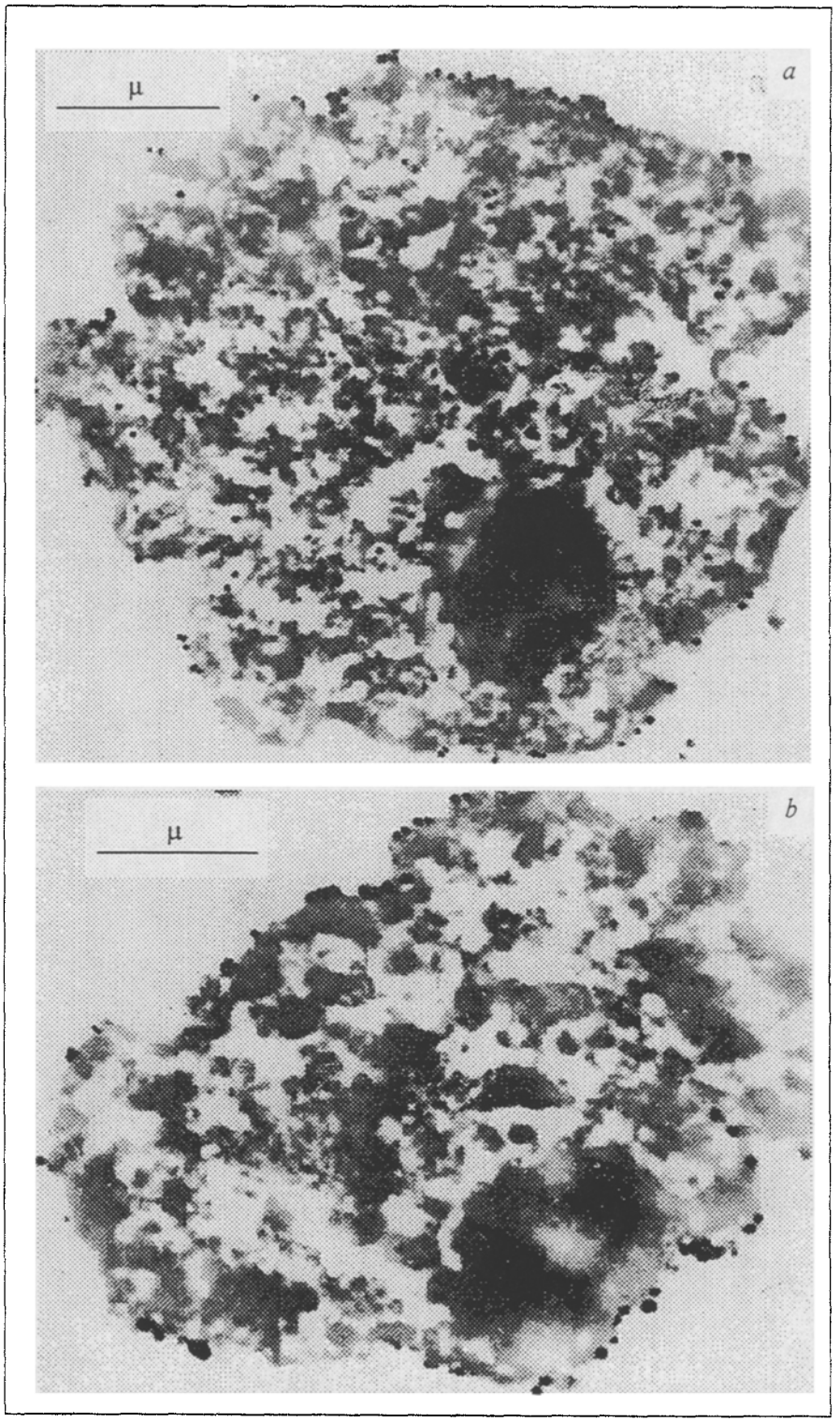

Fig. 3. Electron micrographs of ultrathin sections of mouse ascites hepatoma cell nucleus with a monoclonal antibody conjugated with colloid gold. *a*) with antibody to p260. Gold particles are detected both inside the nucleus and at the periphery; *b*) with antibody to fibronectin. Gold particles are localized only at the periphery of the nucleus. The diameter of gold particles is 40 μ.

REFERENCES

1. T. V. Bul'dyaeva, S. N. Kuz'mina, and I. B. Zbarskii, *Dokl. Akad. Nauk SSSR*, **241**, 1461-1464 (1978).
2. M. Gleiberman, Yu. Yu. Sharovskaya, and E. N. Kudryavtseva, *Tsitologiya*, **30**, 1126 (1988).
3. I. B. Zbarskii and S. N. Kuz'mina, *Skeletal Structures of the Cell Nucleus* [in Russian], Moscow (1991).
4. G. Blobel and R. Van Potter, *Science*, **154**, 1662-1665 (1966).
5. K. Fukuda, and Jen-Fu-Chin, *Arch. Biochem*, **283**, 401-408 (1990).
6. L. Gerace, Y. Ottaviano, and C. Kondor-Koch, *J. Cell. Biol.*, **95**, 826-837 (1982).
7. M. Henderson, T. Isukada, A. V. Gown, *et al.*, *J. Histochem. Cytochem.*, **35**, 419-426 (1987).
8. S. Kaufmann and J. H. Shaper, *Exp. Cell Res.*, **192**, 511-523 (1991).
9. S. N. Kuz'mina, T. V. Bul'dyaeva, L. P. Troitskaya, and I. B. Zbarskii, *Europ. J. Cell Biol.*, **25**, 225-232 (1981).
10. U. K. Laemmli, *Nature*, **227**, 680-685 (1970).
11. H. Nakayasu, K. Ueda, and R. Berezney, in: *Nuclear Structure and Function*, Eds. J. R. Harris and I. B. Zbarskii, New York (1990), pp. 361-364.
12. H. Towbin, T. Strachelin, and J. Gordon, *Proc. Nat. Acad. Sci. USA*, **76**, 4350-4354 (1979).
13. G. Zerlauth, J. Wesierska-Gadek, and G. Sauermann, *J. Cell Sci.*, **89**, 415-421 (1988).