FIBRONECTIN IN CULTURED CELLS OF MOUSE SARCOMAS INDUCED BY FOREIGN BODIES

A. V. Lyubimov and T. G. Moizhess

UDC 616-006.3.04-02:616-003.6]-008.93:577.112.853

KEY WORDS: fibronectin; sarcomas induced by foreign bodies.

Fibronectin is one of the principal adhesive glycoprotein components of the extracellular matrix and is present in various biological fluids. Malignant transformation of connective-tissue cells is often accompanied by complete or partial loss of fibronectin from the cell surface [4, 10]. However, some exceptions are known to this rule, and their number is steadily rising. According to recent data, sarcomas induced by foreign bodies arise from local connective-tissue cells which participate in capsule formation around the foreign body [5].

It was accordingly decided to study the distribution of fibronectin in the cells of these tumors in order to discover whether loss of fibronectin is a marker of the transformed state of cells in such a system.

EXPERIMENTAL METHOD

Tumors were induced in CBA mice by subcutaneous implantation of pieces of polyvinyl chloride film [2, 6]. Cultures of tumor cells were obtained by trypsinization of the minced tumor nodes, freed from necrotic areas. The cells were grown on medium containing 40% Eagle's medium with twice the range of amino acids, 40% lactalbumin hydrolysate, 20% bovine serum, and 200 U/ml of monomycin. Cells of five lines obtained from five cultures grown in this way (PS-4, PS-84, PS-100, PS-103, and PS-130 were used. Low-density 2-3 day and high-density 3-7 day cultures were used for the experiments. Cells of all five lines were highly malignant for syngeneic mice. Primary and secondary cell cultures from connective-tissue capsules surrounding the films also were used. The capsules were taken a long time before the appearance of tumors (2-3 months after implantation) and cells from them were isolated withthe aid of 0.3% collagenase (type I, from Sigma, USA) by the method described in [7]. Capsule cells were cultured under the same conditions as sarcoma cells, for 7-8 days. The distribution of fibronectin on the cell surface was studied bythe indirect immunofluorescence method after fixation of the cells with 4% formalin [1]. The monospecific antifibronectin antiserum was generously provided by Professor A. Vaheri (Helsinki University, Finland). Before use it was absorbed by the culture serum, so that it reacted only with cell fibronectin. The standard controls for immunologic specificity were negative.

EXPERIMENTAL RESULTS

In low-density cultures of all the lines studied fibrils of fibronectin were found on the cell surface (Fig. 1). They were located mainly on the undersurface of the cells, in contact with the substrate, outside the nuclear zone; fibrils also were found on the upper surface (Fig. 1d, e). Usually in low-density cultures there were few fibrils and they were arranged chaotically, sometimes interweaving with each other. In line PS-84, unlike the rest, bundles of thin parallel fibrils were often seen on the under surface, oriented along the long axis of the cell (Fig. 1b). Sometimes the fibrils outlined the stable edges of the cells (Fig. 1d, f) and, in certain cases, they could be seen in the zone of intercellular junctions. Punctate fluorescence also was observed in the region of the endoplasm in lines PS-84, PS-100, PS-103, and PS-130. Specific fluorescence was absent in the lamelloplasm zone in all lines, unlike in normal embryonic fibroblasts [1].

A well-developed network of interwoven fibronectin fibrils was formed on the lower and upper surfaces of the cells in high-density cultures of lines PS-4, PS-100, PS-103, and PS-130 (Fig. 2a, c-e). Sometimes the network had a definitely cellular structure (lines PS-100 and PS-130). In zones in which the cells showed a

Laboratory of Mechanisms of Carcinogenesis, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 9, pp. 339-341, September, 1984. Original article submitted November 1, 1983.

 0007-4888/84/9809-1250$08.50

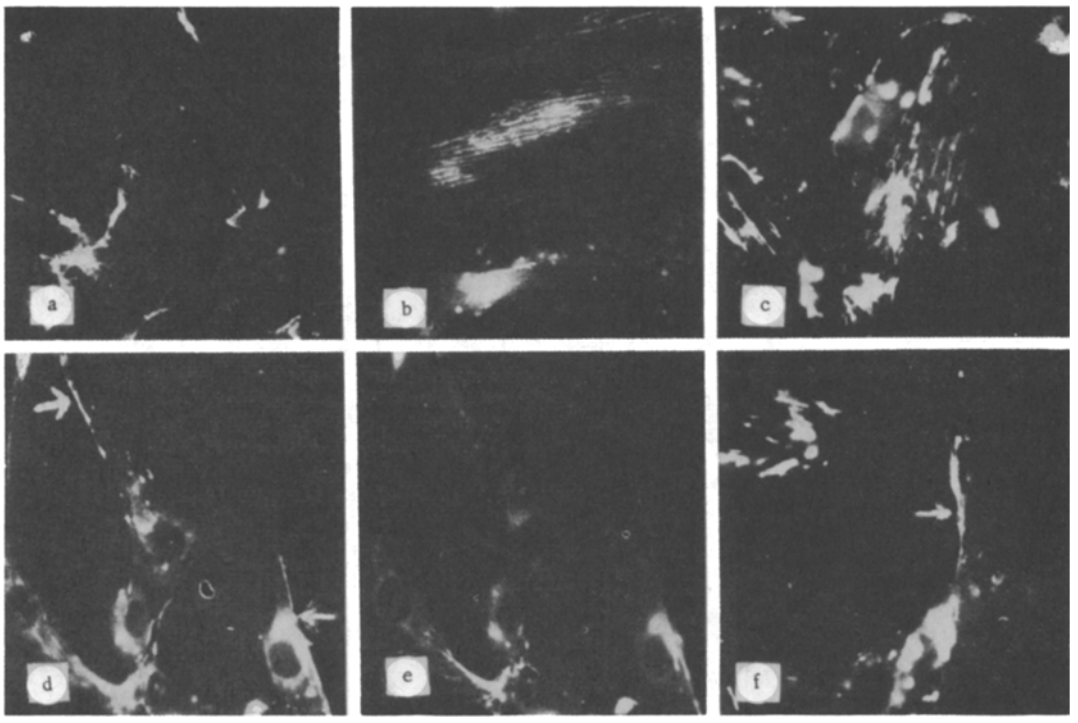

Fig. 1. Distribution of fibronectin in low-density cell cultures of sarcomas induced by foreign bodies. a) PS-4; b) PS-84; c) PS-100; d, e) PS-103; d) focus on lower surface of cells, e) focus on upper surface of cells; f) PS-130; arrows indicate stable edges of cells stained for fibronectin. Indirect immunofluorescence. 580 ×.

definite mutual orientation the fibrils were usually parallel to the long axes of the cells (line PS-100). Sometimes fibrils followed the outlines and long processes of the cells. In the nuclear region, just as in low-density cultures, as a rule fluorescence was absent. In line PS-84, with an increase in density of the culture fibronectin accumulation did not take place: Only single fibrils or small local concentrations of them were visible (Fig. 2b). In dense cultures of capsule cells a well-defined three-dimensional network of interwoven fibronectin fibrils was observed, often with distinct and irregularly shaped spaces between them (Fig. 2f). In less dense areas fibrils were visible in the composition of the fibrous intercellular substance. The fibronectin network in PS-100, PS-103, and PS-130 cultures was well developed, in cultures of capsule cells also, but in line PS-4 the network was rather less well developed. In line PS-84 the fibronectin content was appreciably less than in capsule cells, and it did not form a fibrillary network.

In sarcomas of lines PS-4, PS-100, PS-103, and PS-130 the content of surface fibronectin thus increased with an increase in density of the culture, and at the continuous monolayer stage it formed a dense fibrillary network. A similar result was obtained by the writers previously with a similar line, MTs-1 [3]. Only in line PS-84 was no fibronectin network formed in the high-density cultures and the quantity of fibronectin was less than in other lines of sarcomas and capsule cells. These data are evidence that malignancy of the cells in the cellular system studied does not correlate with loss of surface fibronectin and it cannot be regarded as a reliable marker of the transformed state. Preservation of fibronectin synthesis and deposition in the matrix also was found previously in several human sarcomas [9].

The character of distribution of fibronectin in the form of a three-dimensional network of fibrils was very similar in all lines of sarcomas and capsule cells which accumulate it. This type of distribution is characteristic of fibroblast-like cells [4]. In cells of the vascular endothelium, which are regarded as one of the possible precursors of sarcomas induced by foreign bodies, the distribution of fibronectin in high-density cultures is different: A network is formed only on the lower surface of the cells facing the support [4]. It can be postulated that these sarcomas arose from certain fibroblast-like pretumor cells, possibly identical with cells growing in cultures of capsules. To prove this hypothesis further investigations are necessary, using other specific markers.

After this investigation was completed, we learned of a communication by MacDonald et al. [8], who also showed that fibronectin synthesis and deposition in the matrix were preserved in cell cultures from 13 murine

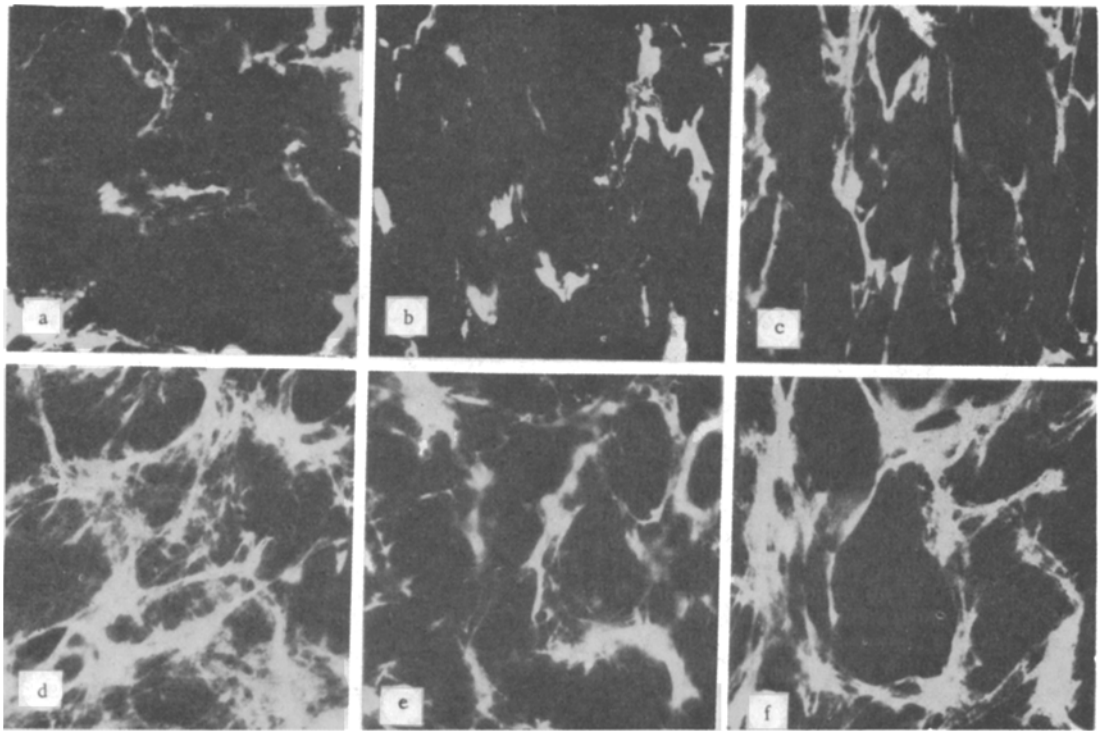

Fig. 2. Distribution of fibronectin in high-density cell cultures from sarcomas and early capsules. a) PS-4, b) PS-84, c) PS-100, d) PS-103, e) PS-130, f) capsule (3 months after implantation, primary culture). Network of fibronectin fibrils absent only in line PS-4. Indirect immunofluorescence. 580 ×.

sarcomas induced by implantation of films made from a vinyl chloride–vinyl acetate copolymer subcutaneously into CBA/H mice.

The authors are grateful to Professor A. Vaheri for providing the antiserum and to Professor Yu. M. Vasil'ev for useful advice.

LITERATURE CITED

1. Yu. M. Vasil'ev and A. V. Lyubimov, Tsitologiya, No. 3, 283 (1983).
2. Yu. M. Vasil'ev and T. G. Moizhess, Int. J. Cancer, 30, 525 (1982).
3. A. V. Lyubimov, Byull. Éksp. Biol. Med., No. 1, 74 (1984).
4. A. V. Lyubimov and A. S. Gleiberman, in: Phenomena of Induction and Differentiation during Tumor Growth [in Russian], Moscow (1981), pp. 171-251.
5. T. G. Moizhess, in: Phenomena of Induction and Differentiation during Tumor Growth [in Russian], Moscow (1981), pp. 70-105.
6. T. G. Moizhess, "Time of appearance, localization, and origin of pretumor cells during experimental carcinogenesis induced by implantation of plastic films," Author's Abstract of Candidate's Dissertation, Moscow (1978).
7. K. H. Johnson, L. D. Buoen, I. Brand, et al., Cancer Res., 37, 3228 (1977).
8. G. C. MacDonald, L. T. Furcht, and K. G. Brand, Proc. Soc. Exp. Biol. (N.Y.), 172, 89 (1983).
9. S. Stenman and A. Vaheri, Int. J. Cancer, 27, 427 (1981).
10. K. M. Yamada, in: The Glycoconjugates, Vol, 3, New York (1982), pp. 331-362.