LITERATURE CITED

1. M. M. Volokhova, V. A. Gubin, N. G. Darenskaya, et al., Problems in General Radiobiology [in Russian], Moscow (1966), pp. 150-162.
2. N. G. Darenskaya, V. S. Kashirin, and S. S. Kuznetsova, Radiobiologiya, 17, No. 1, 67 (1977).
3. Nguen Thyong, "Investigation of neurohumoral regulation of gastric digestion in lower monkeys," Author's abstract of dissertation for the degree of Candidate of Medical Sciences, Sukhumi (1968).
4. L. V. Nikolaevskaya, V. E. Komar, E. A. Zherbin, et al., Radiobiological Approaches in the Diagnosis of Radiation Damage [in Russian], Leningrad (1987), pp. 52-55.
5. I. V. Remizova, Radiobiologiya, 24, No. 6, 804 (1984).
6. L. F. Semenov, Experimental Prevention of Acute Radiation Sickness [in Russian], Leningrad (1967).
7. N. G. Chigareva and V. M. Teslenko, Radiobiologiya, 24, No. 3, 408 (1984).
8. I. L. Mattsson and M. C. Vochmowitz, Radiat. Res., 82, No. 1, 191 (1980).
9. R. R. McGregor, Ann. Intern. Med., 86, No. 1, 35 (1977).

REGULATION OF DNA SYNTHESIS BY FIBRONECTIN AND ITS PROTEOLYSIS PRODUCTS IN SKIN FIBROBLASTS OF HEALTHY DONORS AND PATIENTS WITH SYSTEMIC SCLERODERMA AND RHEUMATOID ARTHRITIS

O. Yu. Abakumova, N. G. Kutsenko, and A. F. Panasyuk

UDC 616.5-004.1-031.81+616.72-002.77-039]-07:[616.5-008.939.633.2-02:616.5-008.93:577.112.853]-092.4

KEY WORDS: fibronectin and its fragments; human skin fibroblasts; DNA; systemic scleroderma; rheumatoid arthritis.

The fibronectins are a family of high-molecular weight glycoproteins which are involved in cellular adhesion and migration, organization of the cytoskeleton, and processes of embryonic development, hemostasis, thrombosis, wound healing and malignant transformation [11]. In addition it is becoming increasingly evident that fibronectin plays a role in the pathogenesis of various diseases [3, 6]. Investigations have demonstrated the role of fibronectin and its fragments as growth factors and regulators of cell growth [4, 7, 9, 10]. It was shown previously that removal of gelatin-binding fragments (gel-fragments) from tryptic digests of the fibronectin molecule leads to marked stimulation of DNA synthesis in fibroblasts, and gel-fragments themselves inhibit DNA synthesis by 50-75% [2].

In the investigation described below a comparative analysis was undertaken of the effect of fibronectin and its tryptic hydrolysis products on DNA synthesis in cultures of skin fibroblasts from patients with rheumatoid arthritis (RA) and systemic scleroderma (SSD), and also of healthy blood donors (HD).

EXPERIMENTAL METHOD

Cell cultures and technique of culture were described previously [1]. The cultures were tested at the 3rd-10th passages in the stationary phase of growth. DNA analysis was carried out, using medium 199 with 0.5% solution of embryonic calf serum. Cells were synchronized in this same medium, cultured for 24 h. ^{14}C-Thymidine (USSR) was used as labeled precursor

Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 12, pp. 678-681, December, 1989. Original article submitted December 25, 1988.

 0007-4888/89/0012-1724$12.50

TABLE 1. Effect of Removal of Gel-Fragments from Culture Medium on DNA Synthesis in Skin Fibroblasts in Culture (culture medium taken without and after treatment of cell monolayer with trypsin; control data taken as 100%)

Skin fibroblasts and culture medium of fibroblasts	Without treatment by trypsin		After treatment with trypsin	
	with gel-fragments	without gel-fragments	with gel-fragments	without gel-fragments
	DNA synthesis, %			
Patients with SSD	124	159	140	644
	91	83	61	770
	283	219	320	419
	127	91	140	230
	—	—	444*	1319
	—	—	203**	664
Patients with RA	72	198	138	1948
	68	83	97	146
	52	161	93	264

Legend. *) Experiment with culture medium of skin fibroblasts from patients with RA; **) with culture medium of fibroblasts from HD.

TABLE 2. Effect of Fibronectin (from different sources) on DNA Synthesis in Skin Fibroblasts from Patients with RA and SSD and from HD. Tryptic Digests and Gel-Fragments of Fibronectin Obtained by Proteolysis of Fibronectin by Trypsin at 37°C for 100 min with Substrate and Enzyme Present in the Ratio of 100:1. Concentration of Fibronectin and Its Fragments on Addition of the Cells with 20 μg/ml. Control Data Taken as 100% (mean results of five or six independent experiments)

Material	DNA synthesis (in %) in skin fibroblasts in culture		
	patients with SSD	patients with RA	HD
Plasma fibronectin	130,40±55,20	82,50±14,95	147,30±35,50
Its tryptic digest	288,25±114,00	156,00±47,85	116,25±24,90
Gel-fragments	81,80±21,80	59,50±11,40	65,66±9,20
Fibronectin from culture medium:			
skin fibroblasts from patients with SSD	290,50±36,35	69,25±5,51	101,67±5,25
its tryptic digest	436,00±70,20	47,00±6,18	88,15±10,12
gel-fragments	37,50±2,48	37,50±8,46	55,22±13,15
skin fibroblasts of patients with RA	339,05±15,94	89,22±13,20	82,17±15,14
its tryptic digest	250,03±75,80	55,16±4,28	35,23±5,18
gel-fragments	71,50±4,48	33,18±5,72	31,14±4,27

and was added 16 h after the beginning of the experiment (labeling time 8 h). The fibronectin and gel-fragments used in the work were isolated from plasma or from the culture medium of the fibroblasts and estimated as described previously [2]. The technique of proteolysis of fibronectin and of treatment of the culture medium also are described in [2]. Fibronectin, its tryptic digest, and the gel-fragments were added to the culture medium in concentrations of 2.5 to 26 μg/ml. Since we propose a possible modification of relations between fibronectin and its fragments, on the one hand, and cells under pathological conditions, not only fibronectin of the plasma, but also fibronectins isolated from the culture medium of the test fibroblasts were studied in these experiments. At the end of the experiment the cells were placed on ice, washed with ice-cold Earle's medium, and then lysed in 0.3M KOH; acid-insoluble material was precipitated with 10% TCA. Determination of radioactivity and of cell protein was described previously [1, 2].

EXPERIMENTAL RESULTS

After removal of fibronectin gel-fragments from the culture medium it was able to intensify DNA synthesis considerably in fibroblasts in culture (Table 1). This was particularly

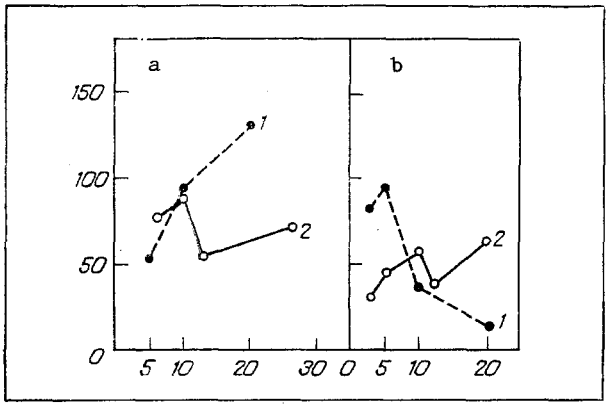

Fig. 1. Effect of various concentrations of fibronectin (a) and of gel-fragments (b) on DNA synthesis in skin fibroblasts from patients with SSD (1) and from HD (2). Abscissa, concentration of fibronectin and of gel-fragments (in μg/ml); ordinate, incorporation of ^{14}C-thymidine (in % of control).

TABLE 3. Dependence of Incorporation of ^{14}C-Thymidine into DNA of Skin Fibroblasts of HD on Addition of Fibronectin of Different Origin and of Dithiothreitol (mean results of three independent experiments)

Material	Incorporation of ^{14}C-thymidine, cpm/mg protein of fibroblasts	
	without dithiothreitol	with addition of 17 mM dithiothreitol
Control	2400	3140
Plasma fibronectin	2565	1702
Fibronectin from culture medium:		
skin fibroblasts of HD	2906	2059
skin fibroblasts of patients with SSD	2069	2988
skin fibroblasts of patients with RA	2171	3494
Tryptic digests of plasma fibronectin without gel-fragments:		
after proteolysis for 10 min	2898	1471
after proteolysis for 100 min	7108	2445

Legend. Fibronectin and its tryptic digests added to culture medium in a concentration of 25 μg/ml.

characteristic of the culture medium of skin fibroblasts from patients with SSD and RA. This considerable stimulation of DNA synthesis may be evidence that either gel-fragments are inhibitors also of growth factors secreted by fibroblasts into the culture medium [8] or, on affinity chromatography, other inhibitors of DNA synthesis, with affinity for gelatin, are removed from the culture medium.

Fibronectin from the culture medium of skin fibroblasts of patients with SSD and RA, in the concentration tested, increased DNA synthesis two-threefold in skin fibroblasts of patients with SSD, and only in these fibroblasts was DNA synthesis also stimulated by tryptic digests of fibronectin (Table 2). The mechanism of this stimulation is not clear; a definite role here may perhaps be played by the higher content of collagen, synthesized and secreted by skin fibroblasts from patients with SSD [5], compared with skin fibroblasts from HD and patients with RA, which may bind the gel-fragment from the tryptic digest of fibronectin and thereby block its inhibitory activity.

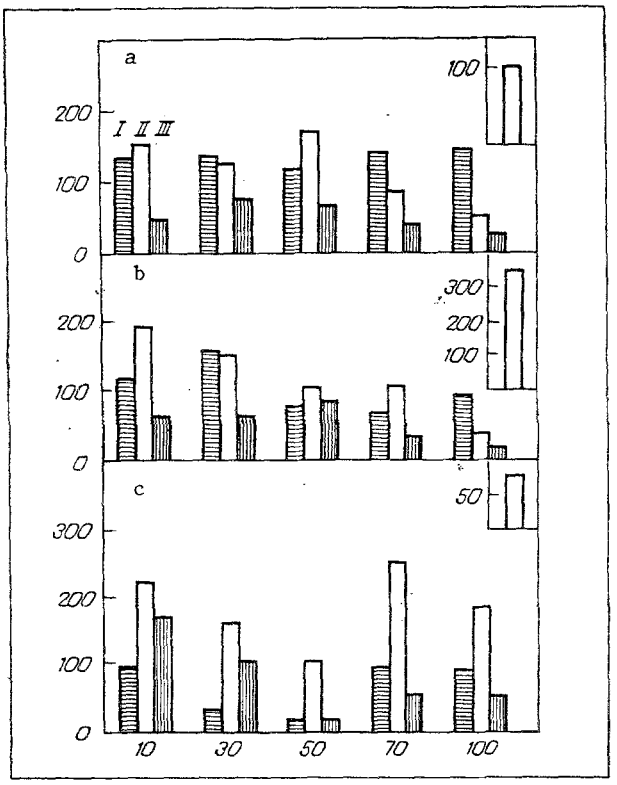

Fig. 2. Incorporation of ^{14}C-thymidine (in % of control) into DNA of skin fibroblasts of patients with SSD (a, b) and HD (c) on addition to culture medium of 10 μg/ml (a, b) or 20 μg/ml (b) of tryptic digest of fibronectin (I) and tryptic digest without gel-fragments (II), and 10 μg/ml (c) and 20 μg/ml of gel-fragments (III). Insets: DNA synthesis (in % of control) on addition of 10 μg/ml (a, b) and 20 μg/ml (b) of fibronectin. Abscissa, duration of proteolysis of fibronectin by trypsin (in min); ordinate, incorporation of ^{14}C-thymidine (in % of control).

Only in skin fibroblasts from patients with SSD was a dose-dependent effect of fibronectin and of its gel-fragment observed relative to DNA synthesis (Fig. 1). The inhibitory action of the gel-fragment began to be manifested when the concentration was about 10 μg/ml.

The size of the fragments formed during proteolysis of the plasma fibronectin was inversely proportional to the duration of proteolysis [2]. As Fig. 2 shows, the size of the fragments obtained determines both the level and even the direction of their action on DNA synthesis. The strongest inhibitory action on skin cells of patients with SSD is manifested by the gel-fragment obtained after protelysis for 70 and 100 min, this effect being dependent on concentration (Fig. 2a, b). In skin fibroblasts from HD, gel-fragments formed during proteolysis for 10 and 30 min did not inhibit DNA synthesis in a concentration of 10 μg/ml, and at all times of proteolysis stimulation of DNA synthesis by the tryptic digest after removal of the gel-fragments was observed. The highest values of inhibition of DNA synthesis by the tryptic digest and gel-fragment were obtained by the use of fibronectin proteolysis products for 50 min (Fig. 2c).

Fibronectin of the plasma not only binds with the surface of human skin fibroblasts in culture, but also is taken up into the extracellular matrix [12, 14]. Disulfide bonds are involved in the binding of fibronectin with cells and collagen, and their blocking reduces this binding sharply [15]. On the addition of fibronectin, isolated from plasma and culture medium of skin fibroblasts of HD, and of its tryptic digests together with dithiothreitol, to the culture medium, a decrease in DNA synthesis was observed (Table 3), i.e., for the mitogenic effect to be exhibited, the plasma fibronectin and cellular fibronectin must be bound with cells. Dithiothreitol not only did not abolish, but actually potentiated the

stimulating effect of fibronectins isolated from the culture medium of skin fibroblasts of patients with RA and SSD.

In a comparison of the data presented in Table 2 and in Fig. 1 and those obtained on skin fibroblasts of patients with SSD, it is clearly evident that to obtain the maximum stimulation of DNA synthesis it is essential to combine fibronectin isolated from the culture medium of skin fibroblasts of patients with RA and SSD and skin fibroblasts of patients with SSD. Tryptic digests of plasma fibronectin have no such sharp stimulating action.

We found previously that DNA synthesis in skin fibroblasts of patients with SSD is about 7 times higher in intensity than in skin fibroblasts from HD [1]. The role of one of the extracellular signals maintaining a state of preparedness of skin fibroblasts of patients with SSD for DNA synthesis may perhaps be played by both fibronectin itself and its fragments, more especially because the quantity of fibronectin in a monolayer of these cells is 2 to 3 times higher than normal [5]. Whereas fibronectin, as it is supposed, is a growth factor belonging to the class of competence factors [4], the presence of a corresponding progression factor, responsible for the switching of competent cells directly to DNA synthesis, in the culture medium may also be postulated.

The authors are grateful to T. V. Usova and E. S. Mogilevskaya for technical help with the work.

LITERATURE CITED

1. O. Yu. Abakumova, A. F. Panasyuk, and N. G. Kutsenko, Byull. Éksp. Biol. Med., No. 2, 156 (1985).
2. O. Yu. Abakumova, N. G. Kutsenko, V. Kh. Mitina, et al., Dokl. Akad. Nauk SSSR, 249, No. 4, 984 (1987).
3. A. R. Abdullin, R. I. Litvinov, O. D. Zinkevich, and I. G. Salikhov, Ter. Arkh., No. 4, 89 (1988).
4. P. B. Bitterman, S. J. Rennard, S. Adelberg, and R. G. Crystal, J. Cell Biol., 97, 1925 (1983).
5. R. B. Buckingham, S. Gay, R. Gay, et al., Proceedings of the 7th European Symposium on Connection Tissue Research, Amsterdam (1982), p. 82.
6. A. J. d'Ardenne and J. O'D. McGee, J. Pathol., 142, 235 (1984).
7. G. de Petro, S. Barlati, T. Vertio, and A. Vaheri, Proc. Natl. Acad. Sci. USA, 78, No. 8, 4965 (1981).
8. D. Gospodarowicz and G. Neufield, NATO ASI Series, H5, 191 (1987).
9. G. A. Homandberg, J. E. Williams, D. Grant, et al., Am. J. Pathol., 120, 327 (1985).
10. M. J. Humphries and S. R. Ayad, Nature, 305, 811 (1983).
11. R. O. Hynes, Harvey Lect., Series 81, 133 (1987).
12. P. J. McKeown-Longo and D. F. Mosher, J. Cell Biol., 97, 466 (1983).
13. D. Mosher, Hepatology, 6, No. 6, 1419 (1986).
14. P. D. M. Pesciotta and D. F. Mosher, J. Cell Biol., 104, No. 1, 121 (1987).
15. D. D. Wagner and R. O. Hynes, J. Biol. Chem., 254, 6746 (1979).