CHANGES IN CHROMATIN STRUCTURE OF FIBROBLASTS IN A PATIENT WITH DOWN'S SYNDROME AND IN HIS MOTHER

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UDC 616.899.65-056.52-055.2-07:616.155.32.018.13:576.315.42

KEY WORDS: Chromatins; fibroblasts; Down's syndrome.

By method of luminescent microscopy with acridine orange the writers previously discovered a clear difference in melting profiles of chromatin from lymphocytes of healthy human subjects and patients with Down's syndrome in short-term (1 h) cell cultures [4-6]. The melting curves of chromatin from intact lymphocytes of most healthy subjects were shown to have maxima (F_{530}) in the regions of 45, 65, 78, 88, and 92°C ($P < 0.01$). Melting profiles of lymphocytes from patients with Down's syndrome are curves with maxima in the regions of 65, 85, 88, and 92°C ($P < 0.01$).

The absence of a decrease in the intensity of fluorescence between 78 and 85°C and also the absence of a maximum of 45°C were attributed to the greater degree of condensation of particular regions of the chromatin complex of the trisomic cells.

The object of the present investigation was to study whether the changes discovered in chromatin structure in persons with an abnormal karyotype are linked with specific features of the reaction of the immunocompetent tissue (lymphocytes) or whether this phenomenon is characteristic of the body as a whole, i.e., of other cells of the given individual. To examine this problem the structure of chromatin in a culture of fibroblasts from a patient with Down's syndrome, his healthy parents, and three other healthy persons (two men and one woman) as the control, was investigated.

EXPERIMENTAL METHOD

A culture of fibroblasts was isolated from material of skin biopsies taken from the patient himself (with Down's syndrome) and his healthy parents. Fragments of skin (0.5 × 0.5 mm) were placed between two coverslips in penicillin flasks in standard nutrient medium with the addition of 20% bovine serum. For direct investigation at appropriate passages a cell suspension with a concentration of 5×10^4 cells/ml was seeded in penicillin flasks with coverslips on the bottom and with fixation of the cells after 48 h. Chromatin from fibroblasts of healthy subjects (two men) and patients with Down's syndrome (two men) from a culture bank (No. 9), of cells grown under standard conditions of culture, was additionally investigated. Parallel with these standard conditions of culture, fibroblasts from the test patient and his parents also were grown in a second group of penicillin flasks, but with the bovine serum replaced by standard nutrient medium containing autologous serum of the test subjects (concentration 20%). Three experiments on each culture were performed at the following passages: from patients with Down's syndrome at the 6th, 7th, and 19th passages, from the parents at the 6th, 7th and 10th, and from the healthy controls at the 8th, 23rd, and 26th passages.

Structural modifications of the chromatin of the fibroblasts under the influence of temperature were investigated by luminescent microscopy with the dye acridine orange (AO). Thermal denaturation of the cells by a modified Ringertz' method [7] was used as the test for the state of chromatin structure. The modifications consisted of removal of formalin as fixative, as being an additional agent causing denaturation that is difficult to control. To prevent renaturation processes, acetone–ethanol (1:1) was used as fixative at temperatures of —5 and

Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 5, pp. 603-606, May, 1980. Original article submitted May 15, 1979.

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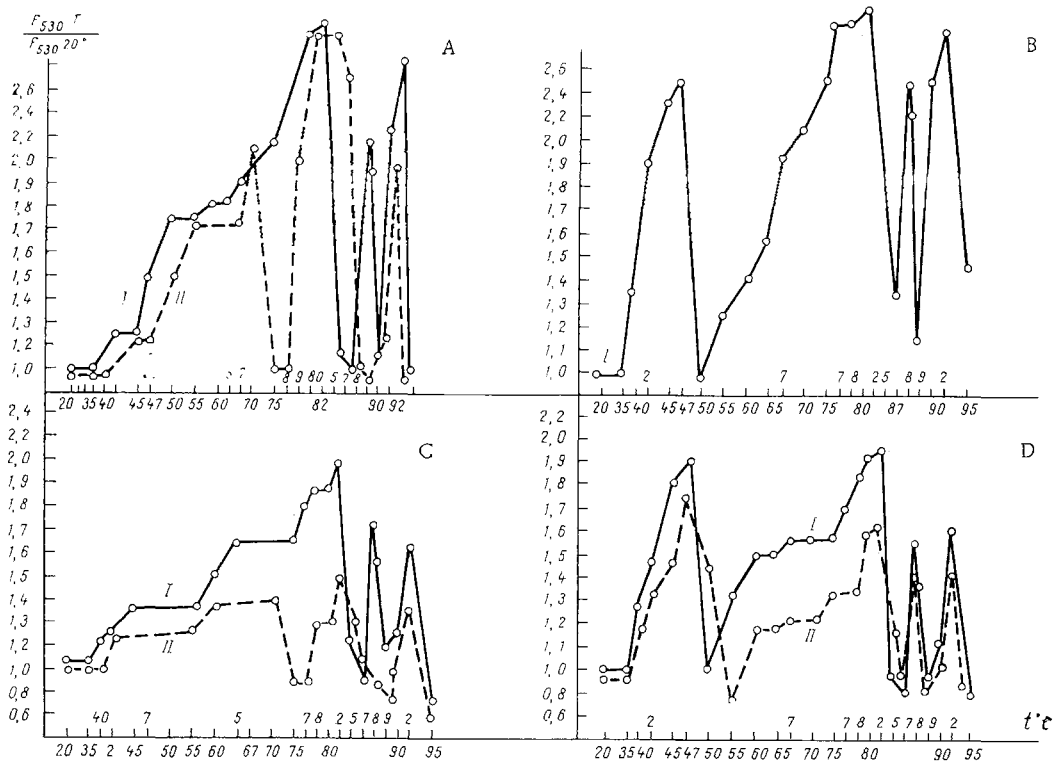

Fig. 1. Melting profiles of chromatin of human fibroblasts during incubation of cells in bovine serum (I) and in autologous serum (II), obtained by luminescence microscopy with the aid of AO (F_{530}). Abscissa, temperature (in °C); ordinate, ratio of intensity of fluorescence of AO at the given temperature to intensity of fluorescence at 20°C

$$\left(\frac{F_{530}\,T^{\circ}}{F_{530}\,20^{\circ}}\,\frac{F_{640}\,T^{\circ}}{F_{640}\,20^{\circ}};\ \text{«}\alpha\text{»}\right).$$

A) Patient with Down's Syndrome; B) control subjects (men); C) proband's mother; D) proband's father.

−7°C, on the assumption that when the holder with the specimens was transferred from the thermostat to the fixative the temperature of the latter would not rise above 0°C. The resistance of fibroblast chromatin to the action of heat was investigated at temperatures of between 25 and 100°C with intervals of 2-2.5°C, and between 70 and 90°C at intervals of 1°C (± 0.1°C). Changes in the structure of chromatin in the cells were tested by a method of luminescent microscopy based on the ability of DNA of chromatin to form complexes with the luminescent label AO. The intensity of luminescence of AO (F_{530}) bound with DNA of fibroblast chromatin was measured on an MSP-0.5 scanning microscope-photometer (Opton). The excitation wavelength was λ = 365 nm; the intensity of fluorescence was determined with the aid of an appropriate filter at λ = 530 nm. Details of the method were described previously [6]. Cells of the same diameter (depending on the size of the probe) were investigated.

Melting profiles of different groups of subjects of all points on the melting curve were compared for the mean intensity of fluorescence and for the parallelness of the change in its intensity. The significance of differences was estimated by a special variant of two-factor dispersion analysis [3]. The significance of the difference in the mean level of the processes was determined as significance of the difference between zero and the mean difference of the partial means, and the significance of divergence from parallelness as the significance of difference between variation of partial differences and random scatter.

EXPERIMENTAL RESULTS

As Fig. 1B, I and Fig. 1D, I show, in response to thermal treatment of fibroblasts from healthy subjects (no sex difference was found in these cultures) and from the proband's

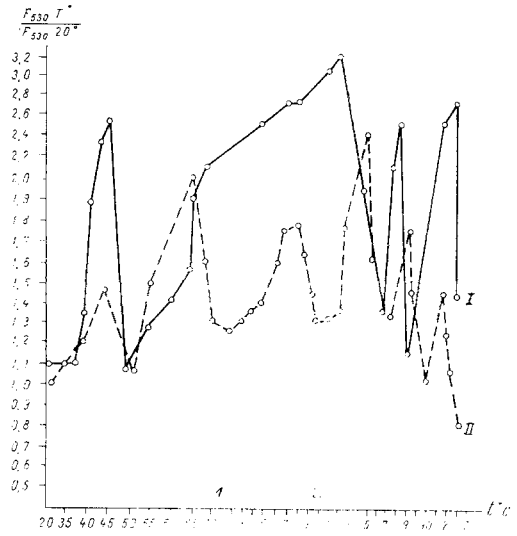

Fig. 2. Melting profiles of chromatin from human fibroblasts (I) and peripheral blood lymphocytes (II) tested by luminescence microscopy with AO (F_{530}). I) Fibroblasts; II) lymphocytes. Abscissa and ordinate, as in Fig. 1.

father the intensity of AO bound with DNA of the chromatin of these cells increased by 2-2.5 times at temperatures of 45, 82, 88, and 92°C ($P < 0.01$), i.e., four maxima were regularly found. The melting profiles of the chromatin of these subjects differed from each other only in the level of fluorescence. It is important to note that temperature transitions of chromatin from fibroblasts incubated both in autologous serum and in bovine serum were indistinguishable from one another in the profiles of the curves; only the general level of fluorescence was reduced along the whole melting curve under the influence of autologous serum. (Fig. 1, II).

A different picture was observed in chromatin from the patient and his mother: The melting profiles of chromatin from their fibroblasts were similar to one another but different from those of healthy subjects and his father, and they also differed depending on the medium in which the cells tested were incubated.

Melting profiles of chromatin from fibroblasts both of the patient himself and of his mother in bovine serum were similar and consisted of curves with three maxima at temperatures of 82, 88, and 92°C ($P < 0.01$) (Fig. 1A and C, I). The peak at 45°C merged with the subsequent maxima.

In autologous serum the melting profiles of chromatin from fibroblasts of the patient and his mother were changed and consisted of curves, also with three maxima, but at different temperatures: peaks at 50-70, 82, and 92°C ($P < 0.01$) (Fig. 1A and C, II).

A fact which deserves special attention is that in autologous serum the melting profiles of chromatin of both fibroblasts and lymphocytes (only of the patient and his mother) were similar to one another and consisted of virtually identical curves (Fig. 1). Differences in the structural organization of the chromatin of fibroblasts of the patient and fibroblasts of patients grown from a culture bank under standard conditions of cell culture were not found. The following conclusions can thus be drawn from the experimental results: The structure of fibroblast chromatin of the patient with Down's syndrome differs from that of healthy subjects and from the proband's own father, but is similar to the structural features of the chromatin of his mother's fibroblasts; structural features of the patient's (and his mother's) chromatin, revealed both in lymphocytes and in cells of different type — a culture of fibroblasts — suggests that changes in the structural organization of the chromatin complex in this disease are characteristic of cells of the other tissues of this individual; a definite part of the changes in chromatin structure is due to the effect of autologous serum, i.e., its external environment which, in this case, is also abnormal [1, 2]; by the method of luminescence microscopy it is possible to detect specific features of the structure of interphase chromatin in cells of different tissues (lymphocytes, fibroblasts) (Fig. 2).

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