INTERPHASE CHROMATIN OF LYMPHOCYTES IN PATIENTS WITH DISTURBED SEX DIFFERENTIATION

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It was shown previously by cytofluorometry with acridine orange (AO) labeling [11] in the writers' modification of thermal denaturation of intracellular DNP [7], that the structure of the interphase chromatin of healthy human lymphocytes exhibits marked polymorphism [6, 7]. In 40% of cases, irrespective of sex, the chromatin melting profiles of the peripheral blood lymphocytes consist of a curve with six (or seven) maxima at certain temperatures. In 60% of cases types of abnormalities regularly repeated in different individuals, the character of which depended on the subject's sex, were discovered. The commonest type of abnormalities in the male control group was absence of the maximum at 85°C, detected on lymphocytes in 25% of cases, and on nuclei of mature spermatozoa (of the same individuals) in 100% of cases [8]. The commonest type of abnormalities for the female control group consisted of fusion of two neighboring maxima into one (25%).

It was shown by the same method that chromatin melting profiles for patients with different types of aneuploidy (Down's and Klinefelter's syndromes) have statistically significant differences between them, and they also differ from those of healthy subjects [7]. It has been suggested that each hereditary disease has its own specific character of structural organization of the chromatin complex of cells chosen for testing.

To settle this problem, and also to study the independent problem of the genesis of diseases with disturbed sex differentiation, it was decided to investigate the structural features of chromatin from peripheral blood lymphocytes of patients with Turner's (aneuploidy for sex chromosomes: 45 XO) and Morris' syndromes (testicular feminization).

EXPERIMENTAL METHOD

Nuclear chromatin of peripheral blood lymphocytes from 20 patients with Turner's and Morris' syndromes aged from 6 to 20 years and from 20 healthy blood donors (as the control), aged from 18 to 25 years, was studied by comparative analysis of melting curves or temperature-dependent structural transitions of the intracellular DNP. To obtain the necessary temperature, a universal U-15 thermostat (East Germany) was used. The accuracy of control was ±0.5°C. Readings were taken at intervals of 1-2°C. Changes in chromatin structure during heating were recorded as the quantity of bound luminescent label — acridine orange (AO). Tests were carried out on cells incubated for 1 h in Eagle's nutrient medium with the addition of 10% autologous serum.

The intensity of luminescence of AO bound with lymphocyte chromatin DNA was measured on the MSP-0.5 microscope-photometer (Opton, West Germany). Luminescence was excited by light with λ = 365 nm and recorded at 530 nm by means of a suitable filter. The instrument, details of the experiment, and method of isolation and incubation of the lymphocytes were described previously [7].

Thermal denaturation of the intracellular chromatin was carried out by the method in [12] in the writers' modification [7]. Melting profiles of lymphocyte chromatin from each

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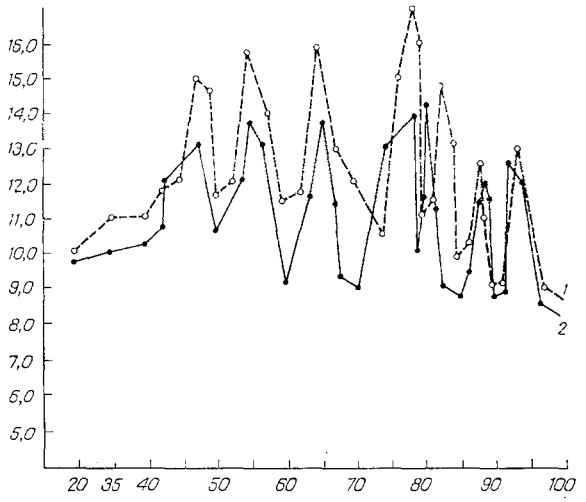

Fig. 1. Melting profiles of interphase chromatin of peripheral blood lymphocytes from patients with Turner's (1) and Morris' syndromes (2), obtained by cytofluorometry with AO. $0.02 \leqslant \sigma \leqslant 0.02$. Abscissa, temperature (in °C). Ordinate, ratio of intensity of fluorescence of AO bound with lymphocyte chromatin at the given temperature to intensity of fluorescence at 20°C (F_o/F_{20}).

individual were compared at all points along the melting curve in relation to mean intensity of fluorescence and parallelness of the change in its intensity. The significance of differences was estimated by a special varient of double dispersion analysis [2, 3]. The significance of differences in the mean level of the processes was determined as the significance of the difference between the mean difference of the partial means from zero, whereas the significance of divergence from parallelness was determined as the significance of the difference between variation of partial differences from random scatter [2].

EXPERIMENTAL RESULTS

Analysis of data for T_{530} of the DNP—AO complex between temperatures of 20 and 100°C showed that chromatin melting profiles of patients with both Turner's and Morris' syndromes are curves with maxima at definite temperatures: 47, 55, 65, 78, 82, and 92°C ($P < 0.01$; Fig. 1).

No statistically significant differences were found between the two clinically and karyotypically different groups of patients, with respect either to parallelness of the melting profiles or intensity of luminescence of the dye (T_{530}) bound with the cell DNP.

The high sensitivity of the method (the least quantity of measurable DNA in chromatin is $5 \cdot 10^{-18}$ mole DNA-PO4 [1]) and the good reproducibility of the results have led some workers to use it to differentiate between different physiological states of the cell, between different phases of the cell cycle [9], and different stages of spermatogenesis [12]. The polymorphism [6] found by the writers previously in relation to specific structural features of cell chromatin of healthy individuals (with a normal karyotype) likewise provide no grounds for disputing the inadequate resolving power of the method for the type of investigation contemplated.

The main difference between the melting profiles of the patients' chromatin and those in the group of "classical" variant of normal healthy human according to this test [6] is absence of the maximum at 85°C in all cases of both groups of patients studied and the appearance of a maximum at 80-82°C. A similar phenomenon of differences in melting profiles of cell DNA, was found previously in the male control group in peripheral blood lymphocyte chromatin in 25% of cases, in mature spermatozoa of these same individuals in 100% of cases [8], and in patients with aneuploidy for the X chromosome (Klinefelter's syndrome) in 100% of cases [7].

What is the reason for the similar structural features of chromosomes in interphase by contrast with the differences existing between them in metaphase in patients with different diseases?

We known that "the transcription activity of the male genome is directed exclusively toward differentiation and the formation of the mature spermatozoon, for unlike the ovum, the spermatozoon does not contribute to the zygote any products of transcribed information" [4].

According to our data, in patients with disturbed sex determination the specific character of organization of the structure of the chromatin complex of lymphocytes is similar to that of ripe healthy human spermatozoa, i.e., a feature of deviations of this test toward the "male" variant is revealed. The changes discovered may perhaps be linked with the absence of correlating action from the ovum (in the period of zygote formation), i.e., absence of the sex determinant of the cytoplasm of the fertilized ovum, since the ovum itself has no specific role in sex cell differentiation [4]. The sex determinant of the cytoplasm of the ovum probably is responsible for linking with sex chromosomes, on the one hand directly (this is an etiologic factor in the karyotypic disturbances in Turner's syndrome) and, on the other hand indirectly, through corresponding receptors. In Morris' syndrome activity of this function of the sex determinant of the ovum is perhaps absent or lost, and this is the cause of the loss of response of the tissue to the androgen [5].

The similarity of the structural features of interphase chromosomes is probably connected with a single pathogenetic mechanism of this group of diseases at the level of the interphase chromatin of diploid cells, which can be identified by cytofluorometry.

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