PHYSICOCHEMICAL PROPERTIES OF DNP SYSTEMS OF LEUKEMIC CELLS WITH DIFFERENT NUMBERS OF CHROMOSOMES

A. I. Svirnovskii, V. V. Nikolaichik, and V. I. Levin

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Investigation of the thermomechanical and disaggregation properties of supramolecular DNP systems isolated from leukemic cells revealed a stronger bond than normally between their components. This property of the DNP structures was independent of the subpopulation to which the leukemic cells belonged and of their number of chromosomes (diploid or hyperdiploid sets), i.e., the change in the properties of the chromatin was more specific for leukemic cells than the numerical chromosomal aberrations.

KEY WORDS: leukemia; chromatin; chromosomes.

The orderly structure of the chromosomes is determined by the supramolecular organization of the principal chemical component of chromatin, DNP. Tumor cells are characterized both by changes in the structure and function of the chromatin and by fluctuations in the number of chromosomes in the set. It is essential in such cases to discover the relationship between these processes and between the intensity of their expression in connection with the relative importance of each process in the formation of the tumor genotype and phenotype. One possible approach to the solution of this problem is evidently to study the characteristics of the macromolecular organization of chromatin in tumor cells of the same tissue specificity, but with different sets of chromosomes.

In this investigation the physicochemical parameters of DNP systems isolated from leukemic lymphoid cells with various disturbances of their chromosome composition were analyzed.

EXPERIMENTAL METHOD

Experiments were carried out on mice of lines AKR and C57BL aged 2 months. Some of the animals were inoculated with leukemia. The AKR mice received an injection of 5 million spleen cells of syngeneic mice with spontaneous leukemia; the C57BL mice received an injection of 5 million spleen cells from animals of the same line with strain La leukemia. The splenic tissue of mice with transplanted leukemia was studied at the time of maximal intensity of the leukemic changes in that organ. To determine the cytological composition of the spleen, no fewer than 500 cells in preparations from each animal were differentiated. The subpopulations to which the normal and leukemic cells of the spleen belonged were determined with the aid of anti-θ serum, obtained by repeated immunization of C3H mice with thymocytes of AKR mice, which showed whether or not they contained the θ-antigen characteristic of T lymphocytes. The number of chromosomes was counted in metaphases after the animals had received two injections of colchicine in a dose of 0.01 ml of the 0.04% solution/g body weight with an interval of 1.5 h. DNP preparations were obtained by first isolating the nuclei [6] by extraction with 0.7 M NaCl. After repeated reprecipitation the DNP was converted into the condensed state [3]. The content of DNA and RNA was determined spectrophotometrically [2] and by color reactions [5, 7]; nitrogen was determined by the micro-Kjeldahl method and protein as described in [9, 12]. The composition of the basic proteins of the DNP was analyzed by electrophoresis [11]. DNP spectra were recorded

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TABLE 1. Physicochemical Properties of DNP Preparations Isolated from Spleens of AKR and C57BL Mice, Intact and Inoculated with Leukemia (M ± m)

Line of mice	Group of mice	Properties of DNP						
		N/P ratio	coefficient of molar extinction	characteristic viscosity (dl/g)		RNA content (%)	relaxation (%)	
				0,7M NaCl	2,0M NaCl		at 20°	at 98°
AKR	Healthy	4,30±0,02	6900±10	24±5	87±3	6,00±0,03	46,5±0,5	42,0±0,9
	With leukemia	4,30±0,02	6800±10	25±2	90±5	7,00±0,04	41,5±0,5	24,5±0,5
C57BL	Healthy	4,25±0,02	6600±30	34±2	95±5	5,50±0,03	54,0±0,1	45,0±0,4
	With leukemia	4,30±0,02	6600±20	35±1	96±3	7,00±0,04	36,5±0,3	25,0±0,4

TABLE 2. Chromosome Composition of Population of Spleen Cells of AKR and C57BL Mice, Intact and Inoculated with Leukemia

Line of mice	Group of mice	number of metaphases	% of metaphase plates (M ± m) with the undermentioned number of chromosomes			
			39	40	41	42
AKR	Healthy	250	7,2±1,6	78,4±2,6	3,2±1,1	
	With leukemia	62	6,8±2,6	73,0±3,8	7,5±2,8	
C57BL	Healthy	132	4,5±1,8	87,2±2,9	2,2±1,3	
	With leukemia	200		12,5±2,1	5,0±1,1	72,5±1,8
	Number of metaphases					

in the ultraviolet region and the spectrophotometric and viscosimetric melting profiles of the preparations were studied. The structural parameters of the three-dimensionally oriented macromolecular DNP systems were judged on the basis of the ability of the DNP to undergo relaxation [3] and disaggregation [1] depending on temperature.

EXPERIMENTAL RESULTS

The results given in Table 1 show that DNP preparations isolated from the spleens of both lines of mice, whether in the soluble or the condensed state, had several similar physicochemical properties. They were indistinguishable as regards both the spectrophotometric and viscosimetric melting profiles, the quantitative and qualitative composition of the basic histone fractions, and the thermomechanical and disaggregation characteristics. As regards the cytological composition of the spleen of the animals, despite its much greater weight (102.4 ± 2.1 mg) and the larger number of cells (121.04 ± 8.84 million) in this organ in the C57BL mice than in the AKR mice (75.6 ± 1.7 mg and 82.53 ± 3.40 million), no significant interlinear differences could be detected. In both cases the proportion of lymphocytes was not below 88-94%. The relative proportions of lymphocytes also were similar as regards both degree of maturity and functional properties. The proportion of T lymphocytes in the spleen of the AKR mice was 26.5 ± 1.2% and in the spleen of the C57BL mice 30.0 ± 1.1% of the total lymphocytes. Meanwhile, in the population of spleen cells of the AKR mice aneuploid cells were found rather more frequently than in C57BL mice (Table 2; $P < 0.05$).

In the spleen tissue of the mice with transplanted leukemia, at the time of investigation no fewer than 90% of the cells were leukemic; infiltration of the spleen in the AKR mice took place on account of cells carrying θ antigen on their surface (their number reached 76.5 ± 2.3%); meanwhile, in the C57BL mice the number of these cells in the leukemic organ was down to 3.0 ± 0.9%. Differences in the chromosome structure of the population of leukemic cells also became clearer between the two forms of leukemia (Table 2). In the C57BL mice the modal clone consisted of cells with 42 chromosomes. The mode for transplantable AKR leukemia remained diploid, although some cells had a near-diploid set of chromosomes.

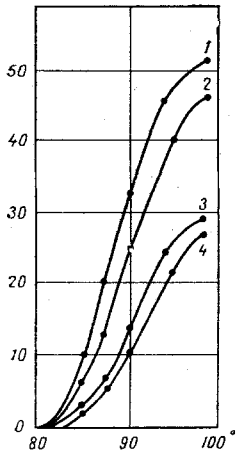

Fig. 1. Further mechanical curves of structural changes in DNP systems isolated from spleen within temperature range of helix–coil transition: 1) DNP system from intact spleen of C57BL mice, 2) from intact spleen of AKR mice, 3) from spleen of C57BL mice with transplanted leukemia, 4) from spleen of AKR mice with transplanted leukemia. Abscissa, temperature of heating; ordinate, degree of relaxation (in %).

DNP preparations obtained from leukemic tissue contained the same basic histone fractions as were present in the chromatin of the spleen cells of healthy animals, their melting profile was the same, and they had other features in common with preparations from normal lymphoid tissue (Table 1). At the same time, the thermomechanical and disaggregation parameters of the chromatin from the leukemic cells were appreciably altered. The kinetics of relaxation of oriented DNP structures was the same for the two forms of leukemia, despite differences in the fluctuations in the number of chromosomes in the set. The course of the thermomechanical curves of structural changes in the DNP systems, especially within the temperature range for the helix–coil transformation (Fig. 1), was evidence of the resistance of the DNP systems from leukemic tissue to deformation and, consequently, of the stronger bond between the components of the chromatin in the leukemic cells. Direct investigation of the strength of the bond between DNA and protein in the condensed DNP structures showed that at 60°C systems isolated from normal lymphoid tissue were more susceptible to disaggregation. For instance, the supernatant after shaking and centrifugation of DNP from the spleen of healthy C57BL and AKR mice was found to contain 17-18.5% of the DNA and 15-18% of the protein contained in the original preparations. The corresponding figures for leukemic tissue from mice of both lines were only 7.0-7.5% DNA and 8.5-9.0% protein ($P < 0.01$), irrespective of the type of leukemia.

Changes in the macromolecular structure of the chromatin in tumor cells, it can thus be assumed, are not necessarily accompanied by structural changes in the chromosomes, whereas numerical chromosomal aberrations, whether present to a considerable or slight degree, suggest a disturbance of the supramolecular organization of the chromatin. This conclusion is confirmed by changes in the properties of chromatin in other tumors also [4]. Consequently, physicochemical modification of the properties of chromatin associated with neoplastic transformation of the cell is a more specific feature than changes in the chromosome number. An essential role in the formation of the material substrate of malignancy is evidently played by the conformational structure of the DNP–protein complex. In the tumor cell the conditions for steric accessibility of the template are different, because of changes in the supramolecular organization of the chromatin [4, 8]. The discovery of a modification of the properties of chromatin provides an explanation for the nonactivation of certain cistrons, functioning in the normal cell, in the tumor cell however, under these circumstances, derepression of individual loci is permitted and does not contradict the possibility of recoding of the genetic activity of the cell. The results are in good agreement with others indicating the possibility of a change in the properties of tumor DNP and of the cells themselves by the action of various factors and with the view that loss of malignancy by cells can be associated with preservation of chromosomal disturbances [14], repression of the virus genome [13], or cell fusion [10].

LITERATURE CITED

1. V. V. Nikolaichik and I. P. Kal'vinkovskii, in: Problems in Experimental Genetics, Minsk (1972), pp. 233-242.
2. A. S. Spirin, Biokhimiya, No. 6, 656 (1958).
3. D. M. Spitkovskii, V. T. Andrianov, and A. N. Pisarevskii, Radiation Biophysics of Nucleoprotein [in Russian], Moscow (1969).
4. D. M. Spitkovskii, Vestn. Akad. Nauk SSSR, No. 1, 29 (1973).
5. K. Burton, Biochem. J., 62, 315 (1956).
6. J. Chauveau, G. Moule, and C. Rouiller, Exp. Cell Res., 11, 317 (1956).

7. V. Endo, J. Biochem. (Tokyo), 67, 629 (1970).
8. J. H. Frenster, in: The Cell Nucleus (ed. by H. Busch), Vol. 1, Academic Press, New York (1974), pp. 565-580.
9. W. S. Groves, F. C. Davis, and B. H. Sells, Anal. Biochem., 22, 195 (1968).
10. H. Harris, Proc. Roy. Soc. London (Biol.), 179, 1 (1971).
11. E. W. Johns, Biochem. J., 104, 78 (1967).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
13. J. A. Macpherson, Proc. Roy. Soc. London (Biol.), 177, 41 (1971).
14. L. Sachs, Harvey Lect., Series 68 (1972-1973), pp. 1-35.