WEAKENING OF DNA-PROTEIN INTERACTION IN CULTURED HUMAN FIBROBLASTS INDUCED BY CHEMICAL MUTAGENS

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Analysis of modification of DNA-protein interaction induced by various pathogenetic agents is an urgent problem in the study of the spectrum of molecular lesions in the genetic apparatus of eukaryotic cells. The writers showed previously that embichin, for example, induces abnormally strong DNA-protein interaction both in isolated chromatin [7] and in chromatin of cultured human fibroblasts [3]. Experiments on isolated chromatin preparations have shown that another type of modification of these interactions may be their weakening, or even dissociation of proteins from chromatin [2]. This type of injury in the cell is virtually impossible to detect by traditional methods of investigation of chromatin in vitro. In recent years the method of nucleoprotein-celite chromatography (NPCC) has been developed in the Laboratory of Tumor Biochemistry, Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, and recommended for use. This technique embodies the principle of separation of nuclei acids and nucleoproteins in lysates of various eukaryotic cells, depending on the strength of binding of DNA with protein of the nuclear matrix [4]. Accordingly, a logical continuation of our research was to use the NPCC method to study the effect of N-nitroso-N-methylurea (NMU) and of prospidine,† which are widely used in medical practice as antitumor agents [8, 10], on DNA-protein interaction in cultured human fibroblasts.

EXPERIMENTAL METHOD

Normal diploid embryonic and postnatal human fibroblasts and human fibroblasts from patients with Down's syndrome were obtained from the cell bank of the Institute of Medical Genetics, Academy of Medical Sciences of the USSR. All cultures were grown on Eagle's medium with 10% human embryonic serum and 10% bovine serum. In the logarithmic stage of growth, some of the cells (control) were labeled with ^{14}C-thymidine, others (experiment), treated with prospidine or NMU, were labeled with ^{3}H-thymidine. After 48 h the control and experimental cells were washed free from radioactive label and the Eagle's medium was replaced by Hanks' solution. Hank's solution containing either NMU or prospidine in a final concentration of 2 mM was added to the experimental cultures. The cells were incubated with the mutagens for 60 min at 37°C. The control cells were incubated under analogous conditions in pure Hanks's solution. In some experiments cells in the stationary stage were treated with the mutagens, for which purpose a monolayer of fibroblasts (10^5 cells/cm^2), after labeling with ^{14}C- and ^{3}H-thymidine, was incubated at 37°C for 3 days with 5% CO_2 on serum-free medium. The stationary stage was monitored visually by noting the absence of mitoses and of a confluent monolayer. The control and experimental cells in all cases were then washed with fresh Hanks's solution, removed from the surface of the flasks with Versene solution, and pooled in equal quantities ($5 \cdot 10^5$ cells in each case). The mixed cells were sedimented from Versene solution by centrifugation (800g, 10 min) and the residue was resuspended in 3 ml of TMK lytic mixture: 25 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$, 10 mM KCl, containing 1% Triton X-100,

†N,N‴-di-(γ-chloro-β-hydroxypropyl)-N,N‴-dispirotripiperazine dichloride.

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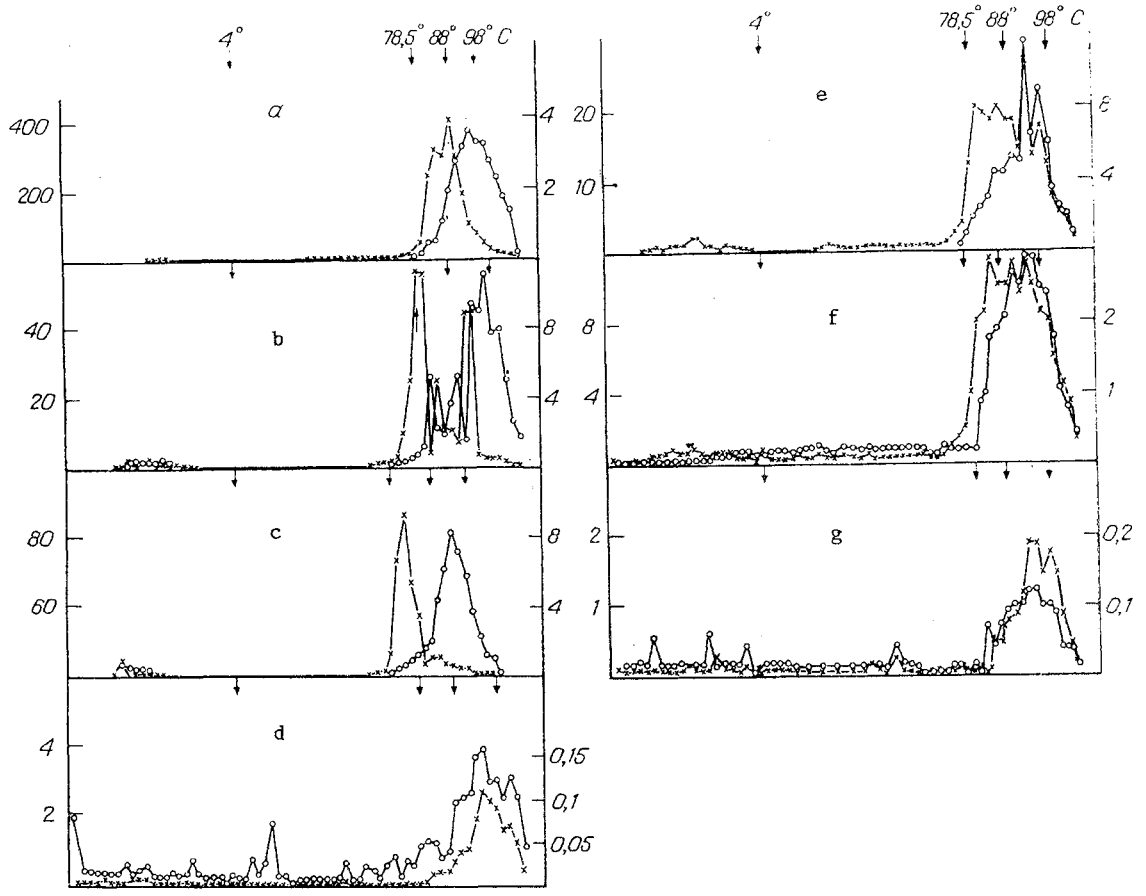

Fig. 1. NPCC of DNA of human fibroblast cultures incubated with NMU (a-d) and prospidine (e-g). Abscissa, no. of fraction; ordinate: on left – radioactivity of ^{3}H-thymidine, in cpm·10^{-3}, on right – the same for ^{14}C-thymidine. Circles – control, crosses – experiment. a) Postnatal fibroblasts, strain 401, 10th passage; b, e) embryonic fibroblasts, strain 814, 15th passage; c, f) postnatal fibroblasts from patient with Down's syndrome, strain 944, 4th passage; d, g) embryonic fibroblasts, strain 814, incubated with agents in stationary stage. Arrows indicate beginning and continuation of temperature gradient.

1 mM phenylmethylsulfonyl fluoride, and 0.1% diethyl pyrocarbonate. NPCC was carried out on Celite 545 (Serva, West Germany) as described previously [4, 5].

EXPERIMENTAL RESULTS

Several workers, using the NPCC method, have shown that at least two types of DNA-matrix complexes may be present in a lysate of certain eukaryotic cells, one of them characterized by weak DNA-protein interaction, so that this complex dissociates at 4°C in a linearly increasing concentration gradient of LiCl and urea, whereas the other is characterized by stronger DNA-protein interactions, which are destroyed in the presence of 4 M LiCl and 8 M urea, but only if the cell lysate and eluting solution are heated to 98-99°C [4, 5]. NPC chromatograms for cultured human fibroblasts under normal conditions and after incubation with prospidine and NMU, respectively, are shown in Fig. 1. For all strains of fibroblasts investigated in the logarithmic stage of growth, no peak of DNA elution was found in the salt gradient at 4°C, i.e., the lysates of these cells did not contain DNA weakly bound with the nuclear matrix. They were characterized only by a "strong" type of DNA binding, since complete elution of DNA took place in the presence of maximal concentrations of salts and after heating the eluting solution to 98°C. After incubation of the cells with NMU and prospidine, DNA likewise was not eluted in a salt gradient at low temperature, but the peak of elution during heating was shifted, in all cases studied, into the region of lower temperatures. This fact is evidence that NMU and prospidine, on penetrating into the cell, destabilize the bond between DNA and the nuclear matrix, since less thermal energy is required in this case to elute the DNA. The "weak" type of DNA-matrix interaction likewise was not observed in a

lysate of fibroblasts taken from patients with Down's syndrome (strain 944 IMG). However, the peak of elution from the lysate of these cells lay approximately 5°C lower than that for healthy human fibroblasts. Incubation of fibroblasts of the 944 IMG strain with NMU caused the maximum of the elution peak to be shifted to a temperature of 81°C, whereas incubation with prospidine shifted it to 83°C. The possibility cannot be ruled out that this was due to an increase in spontaneous injuries to the structure of DNA in fibroblasts from patients with Down's syndrome, as has been demonstrated for DNA of human lymphocytes in the presence of this pathology [1]. Differences observed between cells of strain 944 IMG and normal fibroblasts show that the outlook for the development of these investigations in connection with the further study of the specific character of chromatin organization in cells in this pathology is promising.

It has been shown on human blood lymphocytes [5] and SV-40-transformed cultures of Jungarian hamster fibroblasts [6] that actively proliferating cells also are characterized by only a "strong" type of interaction, which is completely transformed into the "weak" type when the cells pass into the stationary state, and this effect is reversible. In the present experiments, the formation of a "weak" type of DNA-matrix interaction was not found, but after incubation of stationary cells with NMU or prospidine, the peak of DNA elution also was shifted into the 88°C region.

Thus only the "strong" type of interaction between DNA and the nuclear matrix is observed in the human fibroblasts studied in these experiments, irrespective of their proliferative activity. Incubation of these cells with active antitumor agents (NMU and prospidine), one of which has a powerful mutagenic action [10], causes appreciable weakening of interaction between DNA and the matrix. Since the nuclear matrix consists to the extent of about 83-98% of proteins [9], it can be concluded from these results that one type of damage to the genetic apparatus of the cell caused by NMU and prospidine is weakening of DNA-protein interactions. Elucidation of the molecular mechanism of weakening of these interactions will be the subjects of independent investigations.

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