IMMUNOSPECIFICITY OF CHROMATIN NONHISTONE PROTEIN–DNA COMPLEXES IN NORMAL AND NEOPLASTIC GROWTH

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1. Introduction

Increasing experimental evidence indicates that the nonhistone proteins of chromatin are involved in the process of cytodifferentiation and regulation of genetic activity. They were shown to be very heterogeneous and tissue-specific by electrophoretic criteria and the presence of certain nonhistone proteins in reconstituted chromatin was found to be necessary for the in vitro transcription of tissue-specific RNA species [1–4]. Another group of nuclear nonhistone proteins is actively phosphorylated and the extent of the phosphorylation could be correlated to the transcriptional activity of chromatin templates [5–7]. By complement fixation, a mixture of nonhistone proteins and DNA, produced by selective dehistonization of chromatin, is immunochemically tissue specific [8,9]. We report here that the neoplastic transformation changes the immunospecificity of nonhistone protein-DNA complexes in chromatin.

2. Methods

Morris hepatomas 7777, 7787, 7800 and 3924A were transplanted by Dr. H. P. Morris at the Biochemistry Department, Howard University, Washington D.C. and shipped to Houston. The tumors were harvested when reaching approximately 2–3 cm in diameter. The cytochemical and biochemical characteristics of Morris hepatomas are well documented in the literature. Canine transmissible venereal sarcoma was kindly donated by Dr. T. J. Yang, Memorial Research Center and Hospital, University of Tennessee, Knoxville. Experimental hepatomas were produced by feeding Fisher rats with Wayne Laboratory Meal pellets (Allied Mills, Inc.) containing 10% corn oil (Mazola) and 0.06% N, *N*-dimethyl-*p*-(m-tolylazo)aniline which was dissolved in the corn oil [10].

The isolation of nuclei and chromatin was described previously [11,12]. The nonhistone protein–DNA (NP–DNA) complexes were obtained by dehistonization of chromatin in 2.0 M NaCl, 5.0 M urea, sodium phosphate buffer, pH 6.0. Ultracentrifugation of this mixture at 110 000 *g* for 36 hr produced a pellet of DNA and nonhistone proteins leaving histones and about 20% of the nonhistone proteins in the supernatant [13]. The NP–DNA pellets were used for immunization of rabbits according to the schedule described by Chytil and Spelsberg [8]. The antisera were decomplemented by heating at 56°C for 30 min and the γ-globulin fraction of rabbit serum was obtained by ammonium sulfate precipitation and DEAE cellulose chromatography [14]. Lyophilized guinea pig serum complement C′, washed sheep erythrocytes, and rabbit anti-sheep erythrocyte serum were purchased from Capell Laboratories (Downingtown, Pa.). Isotonic Tris–HCl buffer, pH

7.3 (0.1 M NaCl, 10 mM Tris, 0.5 mM $MgCl_2$, 0.15 mM $CaCl_2$, and 0.1% bovine serum albumin) was used for all dilutions.

The immunospecificity of the NP–DNA complexes was determined by the modified microcomplement fixation method of Wasserman and Levine [9,15]. Rat liver and Novikoff hepatoma DNA was isolated according to the previously described [11] modification of the procedure of Marmur [16]. The nonhistone proteins were isolated by dissociation and ultracentrifugation of the NP–DNA complexes in 2.0 M NaCl, 5.0 M urea, 50 mM Tris–HCl buffer, pH 8.0 [17]. For reconstitution, the DNA and nonhistone proteins were dissolved in 2.0 M NaCl, 5.0 M urea, 50 mM Tris–HCl buffer, pH 8.0, mixed together and dialyzed against 0.15 M NaCl, 15 mM sodium citrate pH 7.0 in 5.0 M urea solution. The urea was then removed by rapid dialysis against 0.15 M NaCl, 15 mM sodium citrate solution (SSC). If necessary, the nonhistone proteins or reconstituted complexes were concentrated by ultrafiltration (Amicon UM 2 membrane).

3. Results and discussion

Change in the immunospecificity of chromatin nonhistone protein–DNA complexes by neoplasia is illustrated in figs. 1 and 2. In the presence of normal rat liver NP–DNA antisera, only rat liver chromatin binds the complement significantly. Chromatin preparations from Novikoff hepatoma or from the livers of rats fed 3'MDAB for 111 days (hepatoma) are essentially nonreactive. The same can be seen in a reversed situation (fig. 2) where, in the presence of Novikoff hepatoma NP–DNA antiserum, only the chromatins prepared from various tumors fix the complement extensively. Although chromatins from neoplasms of animal species other than rat are quite immuno-reactive, Novikoff hepatoma (rat) exhibits the highest complement fixation.

The immunoreactivity of chromatins isolated from various Morris hepatomas is compared in fig. 3. It appears that the ability of chromatins to fix complement in the presence of Novikoff hepatoma antiserum increases with the growth rates of individual tumors. The poorly differentiated, fast growing 7777 and 3924A hepatomas are more immunoreactive than

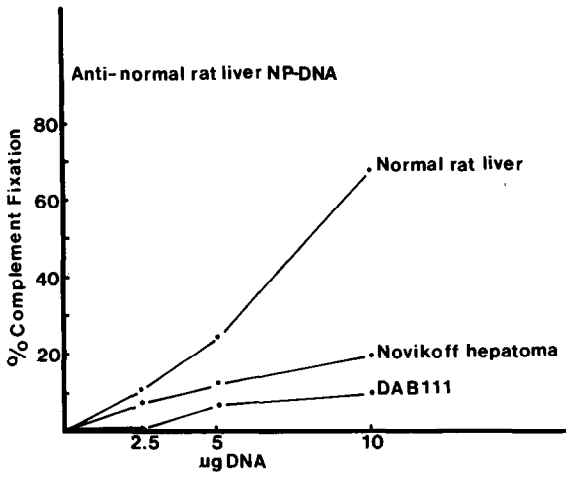

Fig. 1. Complement fixation of chromatin nonhistone protein–DNA complexes from rat liver, Novikoff hepatoma, and livers of Fisher rats maintained on 3' MDAL containing diet for 111 days (DAB 111). The assays were performed in the presence of antiserum against rat liver NP–DNA. The reaction mixture, each containing in a total volume of 0.8 ml various amounts of NP–DNA complexes or chromatin, antiserum (0.1 ml of 200 × diluted rabbit antiserum), and complement (0.2 ml of 50 × diluted guinea pig serum) were incubated overnight at 2–4°C. After incubation, 0.2 ml of activated sheep erythrocytes were added, and the mixture was incubated at 37°C for 20 min. The extent of hemolysis was determined by measuring the absorbancy at 413 nm. All experimented points were corrected for anticomplementarity.

the better differentiated and slow growing 7800 and 7787 tumors.

The immunochemical tissue specificity can be transferred by reconstituting nonhistone proteins from one tissue to the DNA from another tissue of the same species. Fig. 4 illustrates such a transfer between Novikoff hepatoma and normal rat liver. The nonhistone protein fraction NP was isolated from the NP–DNA complexes (ultracentrifugation in 2.5 M NaCl–5.0 M urea – 50 mM Tris–HCl buffer, pH 8.0) and reconstituted with the DNA isolated from the opposite tissue (i.e. normal rat liver NP with Novikoff hepatoma DNA and Novikoff hepatoma NP with normal rat liver DNA). As can be seen, the immunospecificity of the resulting complex was determined by the nonhistone protein (NP) donor tissue.

Chytil and Spelsberg [8] injected rabbits with

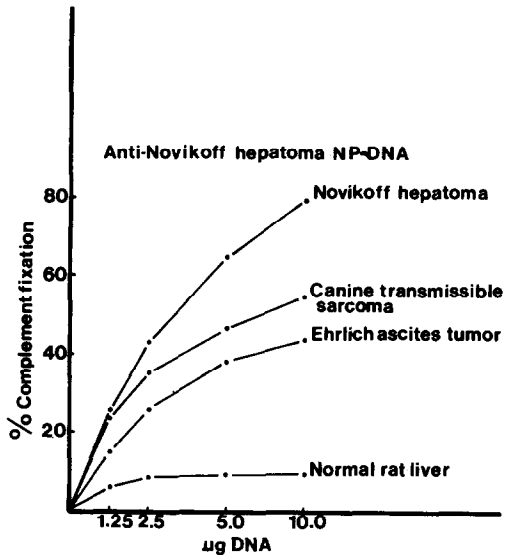

Fig. 2. Complement fixation of chromatin nonhistone protein–DNA complexes from Novikoff hepatoma, canine transmissible veneral sarcoma, Ehrlich ascites, and normal rat liver in the presence of antiserum against Novikoff hepatoma NP–DNA. All experimental points were corrected for anticomplementarity.

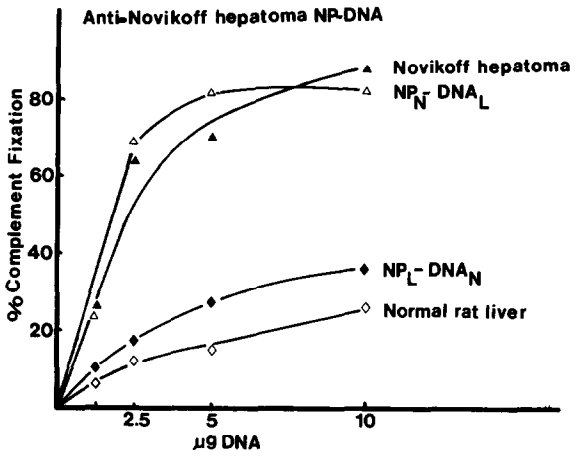

Fig. 4. Complement fixation of normal and reconstituted NP–DNA complexes from rat liver and Novikoff hepatoma in the presence of antiserum against Novikoff hepatoma NP–DNA. All experimental points were corrected for anticomplementarity. (▲——▲) Novikoff hepatoma chromatin (native); (△——△) reconstituted complex of Novikoff hepatoma NP and normal rat liver DNA (NP$_N$–DNA$_L$); (◇——◇) normal rat liver chromatin (native); (◆——◆) reconstituted complex of rat liver NP and Novikoff hepatoma DNA (NP$_L$–DNA$_N$).

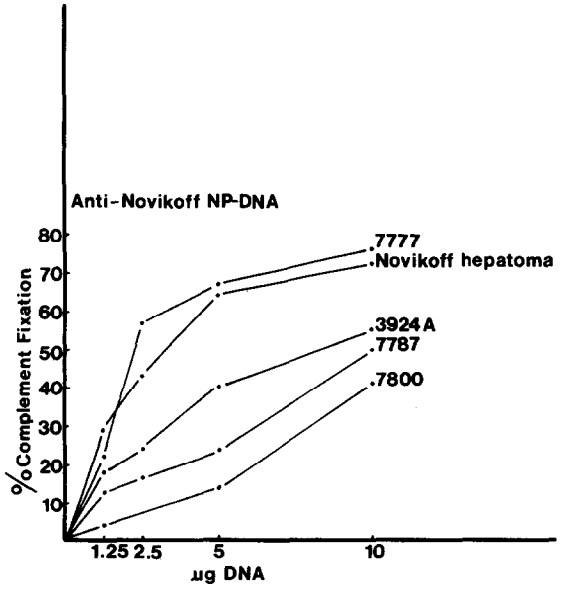

Fig. 3. Complement fixation of chromatin nonhistone protein–DNA complexes from Novikoff hepatoma and Morris hepatomas 7777, 7787, 7800 and 3924A in the presence of antiserum against Novikoff hepatoma NP–DNA. All experimental points were corrected for anticomplementarity.

DNA and nonhistone protein mixture from oviduct and other tissues of chickens and found that these macromolecules elicited the formation of tissue specific antibodies. Their findings were confirmed by Wakabayashi and Hnilica [9] who reported that the immunospecificity of the nonhistone proteins in chromatin described by Chytil and Spelsberg [8] is the result of tissue specific complexes between a fraction of nonhistone proteins and homologous DNA. There was a considerable difference between the immunospecificity of NP–DNA complexes from rat liver as compared with rat hepatoma [9], or between normal and transformed cells in tissue cultures [18]. As is shown in figs. 1 and 2, the immunospecificity of tumors is considerable when compared with a normal tissue (liver) but only minimal when compared with other tumors. Indeed, as illustrated in fig. 2, the animal species of the host seems to play little role in the immunochemical specificity of its NP–DNA complexes. Although mouse is phylogenetically closer to the rat, there is more immunological similarity between tumors from rat and a dog.

Because individual tissues of an animal species differ considerably in the immunochemical properties of their NP–DNA complexes, it appears that the macromolecules interacting with the DNA in such complexes may play an important role in cellular differentiation. Comparison of the immunochemical properties of chromatin from several Morris hepatomas supports this possibility. The more malignant (fast growing and less differentiated) tumors were strongly antigenic as compared with the slow growing and less differentiated hepatomas, bearing the less distinguished phenotype of malignant neoplasms (fig. 3). That the antigenic specificity of NP–DNA complexes depends on their protein components and not the DNA is shown in fig. 4, which compares NP–DNA complexes reconstituted from nonhistone proteins or DNA isolated either from normal rat liver or Novikoff hepatoma. However, more detailed studies are necessary of chromatin nonhistone proteins which can form immunochemically tissue-specific complexes with homologous DNA before they can be assessed any definite biological or gene regulatory functions.

Acknowledgements

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