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INDIRECT CHROMOSOMAL FLUORESCENCE WITH ANTIBODY TO 7,12-DIMETHYLBENZ(a)ANTHRACENE

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Summary

Indirect immunofluorescent technique was applied to acid-alcohol fixed metaphase chromosome preparations from rats treated with the carcinogen, 7,12-DMBA.

Introduction

In order to ascertain the mechanism of polycyclic aromatic hydrocarbon (PCAH) carcinogenesis, investigations involving purely chemical (1) and immunological (2) methods have been employed. Initially the activated moiety must be entrained by some putative target, DNA (3), and then lead inexorably to transformation.

Examination of the aberrant DNA remains a considerable obstacle. A reagent has been prepared by immunization of rabbits with a DMBA bovine serum albumin conjugate to render the elusive carcinogen-DNA conjugate amenable to scrutiny.

Materials and Methods

Antigen was prepared by the mixed anhydride method (4). DMBA-10-succinate was prepared from the phenol (5).

Three New Zealand white rabbits were immunized initially with 6 mg of BSA-DMBA, and 9 mg of antigen at 8th and 10th week. The animals were bled 7 days after the last booster injection. Pooled serum was decomplemented and the IgG fraction isolated by ammonium sulfate precipitation and cellulose elution.

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Marrow was obtained from the femur of a male Long-Evans rat treated with a 50 mg/kg lipid emulsion of DMBA 5 hrs prior to metaphase arrest with colchicine and sacrifice at 6 hrs.

Chromosomes were prepared by hypotonic treatment with 0.075 M KCl, fixation with 1:1 methanol: acetic acid and air dried.

Photooxidation with methylene blue (6) at 65° C for 7 hrs removed histones and denatured DNA.

Anti-DMBA was applied to slides for 20 min and then rinsed with PBS. Fitc-labelled goat anti-rabbit IgG (Miles) was subsequently applied and again rinsed after 20 min.

Chromosome clusters were observed through a Zeiss universal fluorescent microscope with barrier filters 41 and 44 and photographed on Kodak Ektachrome 200 color film.

Results and Discussion

Chromosomes of cells from rat bone marrow appear to bind a considerable amount of PCAH. Overall, the metaphase figures are rather uniformly stained. The extent of chromosomal modification depicted in Figure 1 reflects numerous binding modalities. It is doubtful that a cell so saturated with PCAH would be viable. The lesser, differentially, stained figures were not as numerous. The use of methylene blue was an unobtrusive variable. Fluorescence was neither observed prior to thermal denaturation nor after ultraviolet irradiation or fixation without treatment. Nuclear staining was also seen to varying degrees. After treatment the mitotic figures did not stain with Giemsa, even after prolonged exposure to the dye.

The reagent prepared here demonstrates a potential usefulness as an immunosorbent (7). Coupled with sedimentation procedures (8) large chromosomes can be isolated and analyzed for frequency of modification. In the rat the number two chromosome (9, 10) and various markers derived from it are at times twice the size of even the largest chromosome.

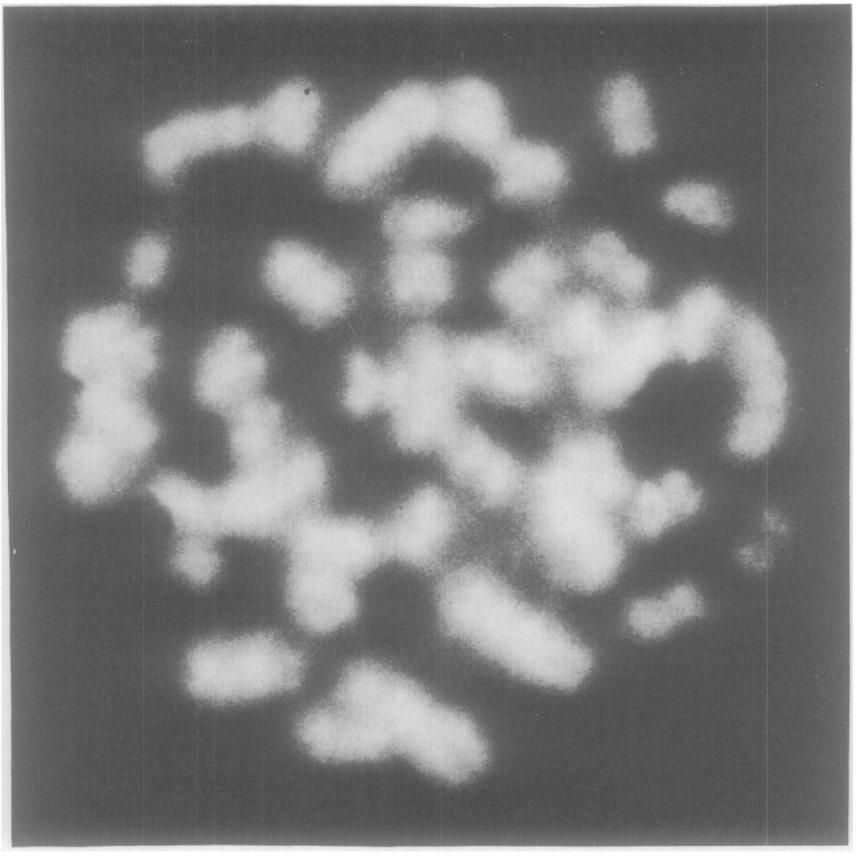

Fig. 1. Early incorporation of carcinogen stained by indirect immunofluorescent technique (x1000). Segmented morphology akin to R-banded chromosomes is expected after photooxidation (6).

Isolation of chromatin derived from specific chromosomes would facilitate further research concerning the chemical nature of PCAH carcinogenesis. If the covalent binding of PCAH's to other macromolecules follows this pattern, then suitable modification of this technique will find many applications.

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