Journal of Cellular Biochemistry 22:245–249 (1983)

Nuclear Protein Changes Following N,N-dimethylformamide (DMF)-Induced Maturation

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A human colonic carcinoma cell line was exposed to a nontoxic concentration of N,N-dimethylformamide (DMF) for 2 wk. Nuclear proteins were isolated from control and treated cells and compared by sodium dodecyl sulfate (SDS) electrophoresis. Qualitative and quantitative differences were observed. Metabolic labeling with tritiated leucine demonstrated qualitative variation between control and treated cells.

Key words: differentiation, nuclear proteins, maturation, DMF, polar solvents

The role of nuclear nonhistone proteins on DNA transcription is unclear. There is evidence that the composition of nonhistone proteins varies within a tissue depending upon the degree of maturation. Other evidence implies that malignant transformation may similarly be accompanied by changes in nuclear protein content. Qualitative changes in nonhistone composition have been reported between normal liver and Novikoff hepatoma, and between murine normal colon and dimethylhydrazine-induced colonic tumors [1, 2]. These results suggest differences in the regulation of gene expression between normal and malignant cells and allude to the possibility that nuclear protein differences may exist between pathologically similar tumors which vary in their degree of aggressiveness. If these alterations exist, they may prove to be clinically significant.

A variety of reagents have been used to alter the maturational status of malignant cells [3–7]. In vitro treatment of malignant cells to polar solvents typically induces a more benign and more differentiated phenotype [4, 5, 7]. While numerous molecular alterations are known to occur during polar solvent exposure, we were most concerned with demonstrations of nuclear protein perturbations. Boffa et al [8] showed that sodium butyrate induced hyperacetylation of the core histones. Administration of dimethyl sulfoxide (DMSO) has been associated with an increased frequency of single-strand DNA breaks, suggesting solvent-mediated perturberations in DNA-protein interactions [9].

Received May 18, 1983; accepted August 19, 1983.

Continuous exposure of colonic carcinoma cells to DMF results in a more normal and more differentiated phenotype [4, 5, 7]. The present study reports the effects of DMF treatment upon the nonhistone nuclear proteins of a human colonic carcinoma cell line.

METHODS

A human colonic carcinoma cell line, obtained from M. Brattain, was grown in McCoy's enriched media containing 10% fetal calf serum [3,10]. A portion of the cells was cultivated in 1% DMF for 2 wk prior to experimental usage. Nuclei were isolated as described by Mullbacher and Ralph [2]. Nuclear proteins were extracted as described by Boffa et al [2]. Briefly, acid soluble proteins were removed by two extractions with 0.25 N HCL. The remaining proteins were resuspended in 0.1 M sodium phosphate (pH 7.4) containing 6 M urea, 0.4 M quanidine-HCL, 0.1 μg phenylmethylsulfonyl fluoride, 0.1% mercaptoethanol, and stirred overnight at 4°C. DNA and protein were separated by centrifugation at 100,000 g for 20 hr. Proteins were dialized against SDS buffer and separated by SDS electrophoresis as described by Weber [12]. Both control and DMF-treated cells were pulsed for 20 hr with 15 μCi tritiated leucine. Gels containing radiolabeled samples were sliced into 1-mm sections and the radioactivity determined in a scintillation counter.

RESULTS

Exposure of the malignant cells to 1% DMF resulted in decreased saturation density, increased doubling time, and inhibition of growth in semisolid medium. These results are similar to the effects of polar solvents on colonic carcinoma cells

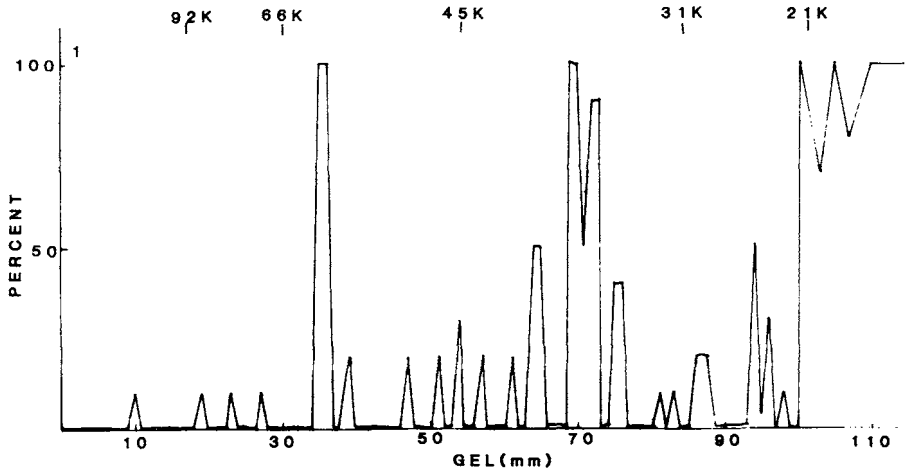

Fig. 1. Nuclear nonhistone proteins were isolated from a human colonic carcinoma cell line and separated by SDS electrophoresis. Gels were stained with Coomassie blue and scanned with a densitometer. Data is expressed as percent absorbance relative to the highest peak of absorption. Bars indicate the position and molecular weight (kilodaltons) of protein standards which were co-electrophoresed. The profile obtained from DMF-treated cells was similar but lacked stained bands with molecular weights of 117, 90, 76, 73, 59, and 50K.

reported by others [4, 5, 7]. DMF treatment did not affect the tumorigenicity of the cell line in nude mice. SDS electrophoresis of the nuclear proteins demonstrated qualitative differences between control and DMF-treated cells (Fig. 1). Stained bands with molecular weights of 117, 90, 76, 73, 59, and 50K were observed only in control cells. DMF-treated cells did not contain uniquely stained bands. The most intense staining for both control and treated cells occurred as an indistinct zone with which a commercial preparation of protamines coelectrophoresed. Boffa et al [1] reported that urea extracts of nuclear proteins from dimethylhydrazine-induced colon tumors contained prevalent bands with molecular weights of 44 and 62K, and that these bands were absent or reduced in normal colon nuclei. The malignant cells used in this study similarly demonstrated intense staining at 44 and 62K. DMF-treated cells demonstrated minimal staining at these weights. Electrophoresis following 20-hr radioisotope incorporation demonstrated simple profiles (Fig. 2). Control cells contained multiple peaks in the protamine region and a minor peak with a molecular weight of 64 K. DMF-treated cells contained a single radiolabeled peak in the protamine region. Extending the chase period to 5 days following the 20-hr pulse with leucine resulted in a more complex profile in the control cells while the DMF treated cells were relatively unaffected (Fig. 3).

DISCUSSION

Nuclear proteins are of critical importance in maintaining DNA structure and in the regulation of gene expression. It is believed that alterations in nuclear protein composition accompany changes in cellular maturation. Therefore the concept of neoplasia as an epigenetic disorder is supported by demonstrations of qualitative variation in the nuclear protein content of normal and malignant cells [1, 2]. Polar solvents have been used to induce a more mature phenotype in a variety of malignant

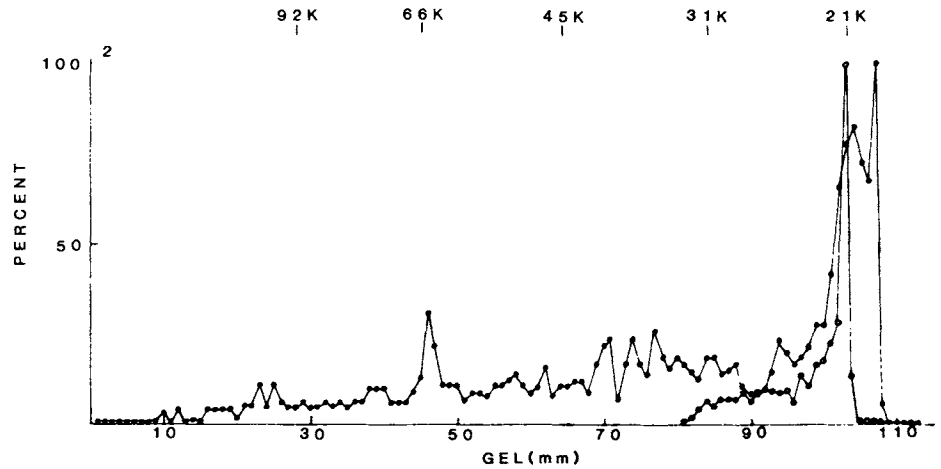

Fig. 2. Control and DMF-treated cells were pulsed for 20 hr with tritiated leucine prior to nuclear protein isolation. Proteins were separated on SDS gels. The gels were cut into 1-mm sections and radioactivity determined with a scintillation counter. Data is expressed as a percentage relative to the highest peak of radioactivity. Bars indicate the position and molecular weight (kilodaltons) of protein standards which were coelectrophoresed. Control cells, ●——●; DMF-treated cells, ○——○.

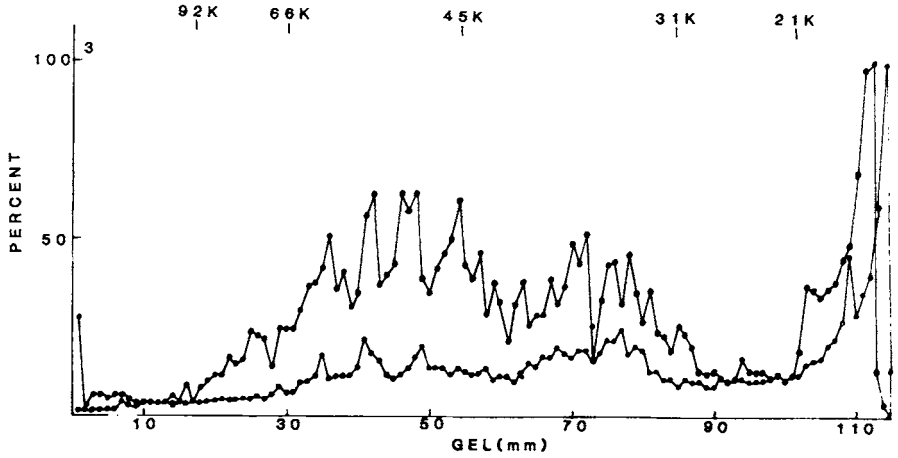

Fig. 3. Cells were pulsed for 20 hr with tritiated leucine 5 days prior to nuclear protein isolation. Method of analysis is the same as for Figure 2. Control cells, ●——●; DMF-treated cells, ○——○.

cells. It is now known that several of these solvents cause modifications of specific nuclear proteins or perturb protein-DNA interactions [8, 9]. DMF has been frequently used to induce a more benign phenotype in colonic carcinoma cells, but its effect on nuclear protein content had not been investigated [4, 5, 7].

Urea extracted material from the control cells demonstrated a relatively simple electrophoretic profile. DMF treatment resulted in a similar electrophoretic profile in which there was a reduction or loss of several stained bands. DMF-induced simplification was similarly seen in the profiles measuring isotopic incorporation. It is tempting to speculate that the additional bands present in the control cells are proteins involved with transcriptional deregulation of the more normal phenotype exhibited by DMF-treated cells. However, there is no evidence of a direct relationship between the loss of certain protein species and the appearance of any of the biological traits. The different radiographic profiles observed with the extended chase similarly suggests that the increased complexity of the control cell proteins is evidence of synthetic variation and not merely an artifact of kinetic differences in cell metabolism. The profiles obtained from the control and more differentiated DMF-treated cells suggest the potential utility of nuclear proteins as tumor specific markers or as indices for the degree of differentiation.

ACKNOWLEDGMENTS

This work was supported by grant PDT-109B from the American Cancer Society and NIH grants CA21520 and CA29495.

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