**ABSTRACT**

The cell surfaces of human colon cancer cells before and after exposure to N,N-dimethylformamide (DMF) were probed using radioiodination and immunofluorescent labeling techniques. Growth of the human colon carcinoma cell line HCT MOSER in DMF-supplemented culture medium resulted in monolayer culture growth and marked cell morphology alterations consisting of cellular flattening and elongation. Accompanying the morphology alterations were distinct changes in the cell surface protein composition as determined by 125I labeling and electrophoresis. The cell surface changes associated with growth of HCT MOSER cells in the presence of DMF were dependent upon time of exposure to DMF and DMF concentration. Furthermore, removal of DMF-treated HCT MOSER cells from DMF-containing growth medium caused reversion of both cell morphology and cell surface composition to a state comparable to that of cells not exposed to DMF. The HCT MOSER cell surface alterations produced by DMF included a reduction of radioiodinated surface proteins with molecular weights of 87,000, 120,000, and 180,000 and an increase of a 125I-labeled surface protein with a molecular weight of 200,000-250,000. Appearance of a surface protein of approximately 200,000 molecular weight and assumption of a fibroblast-like morphology by DMF-treated HCT MOSER cells suggested that this approximately 200,000 molecular weight material might be fibronectin. Immunofluorescent labeling with anti-human fibronectin showed that HCT MOSER cells grown in DMF did manifest an anti-fibronectin immunoreactive material that was only transiently associated with the cell surface before being released. DMF-treated HCT MOSER cultures continued to express surface carcinoembryonic antigen, indicating that the presence of material immunoreactive with anti-human fibronectin was not secondary to proliferation of a contaminating fibroblast population. The response of HCT MOSER cells to DMF paralleled in many ways that previously reported for methylcholanthrene-transformed AKR-2B (AKR-MCA) fibroblasts. However, unlike AKR-MCA cells, HCT MOSER cells did not exhibit an increase in 3H incorporation per mg DNA as a function of time of exposure to DMF, which suggests that the surface protein with a molecular weight of approximately 200,000 induced by DMF was not retained on the cell surface.

**INTRODUCTION**

N-Methylformamide has been identified as a compound able to induce differentiation of cancer cells in vitro and has been evaluated for therapeutic potential in Phase I clinical trials (1, 2). N-Methylformamide is the product of DMF metabolism in humans (3). The maturation-promoting capabilities of the polar solvent DMF have been extensively studied in vitro. Dexter and coworkers (4, 5) have shown that human colon cancer cells grown in the presence of DMF manifest reduced tumorigenicity and clonogenicity in soft agar and exhibit increased expression of CEA and decreased expression of tumor-colonic mucoprotein antigen and H-gene determinant. The differentiation-inducing effects of DMF are not limited to colon cancer cells. DMF has been demonstrated to promote differentiation in Friend murine erythroleukemia cells (6), human HL-60 promyelocytic leukemia cells (7), and murine rhabdomyosarcoma cells (8).

Cell surface proteins have been associated with the malignant biological cell properties of various cancer cell types as well as biological functions of normal cells (9-14). The cell surface tends to reflect intracellular and environmental changes in a dynamic manner (15). According to Moscona (16) the cell surface is normally involved in control of cell differentiation and cell replication by functioning as an interface for signals from the outside to the inside of the cell. Furthermore, various extracellular factors produce changes in the cell surface which, in turn, cause changes in intracellular processes.

In addition to the association of the cell surface membrane of nontransformed cells with such cellular processes as contact inhibition, immune response, regulation of cell proliferation, and differentiation (17-21), the cell surface is also related to certain biological properties of cancer cells. Nicolson (22) has reviewed evidence suggesting that the cell surface is involved in metastasis and evasion of host-mediated immune destruction of tumor cells. Cell surface characteristics have been found to distinguish human colon cancer cell lines that manifest different degrees of aggressiveness in vitro (23, 24). Baylin et al. (9) have shown that human small-cell and nonsmall-cell lung cancer can be distinguished by cell surface phenotype. Alterations in the cell surface have been demonstrated to occur concomitant with drug resistance in cancer cell systems (25, 26).

Although the mechanism of action of differentiation-inducing agents has yet to be clearly elucidated, there is evidence that the cell surface membrane is involved in the differentiation process produced by certain compounds. For example, retinoids have been hypothesized to exert their maturation-promoting actions in a manner analogous to steroid hormones, i.e., retinoids bind to a cytoplasmic protein, enter the nucleus, and then affect gene expression (27-29). However, Yen et al. (30) have shown that interaction of retinoic acid limited to the cell surface membrane is sufficient to elicit differentiation and G1/0-specific growth arrest of HL-60 cells. Lotan et al. (31) have proposed that the cell surface changes produced by growth of the S91 murine melanoma cell line in retinoic acid may be responsible for subsequent growth inhibition. These results suggest that alteration of the cell surface is the mechanism by which some differentiation agents act.

We have previously demonstrated that cell surface changes are associated with transformation of the putatively normal AKR-2B mouse embryonal fibroblast line by chemical and biological agents (32). Furthermore, we have documented that
cell surface alterations correlate with other phenotypic parameters in indicating that malignant, AKR-MCA cells exposed to DMF differentiate and assume the characteristics of nontransformed AKR-2B cells (33). The next phase of our program involved examining the cell surface effects of DMF on a spontaneously arising solid human cancer.

Colorectal carcinoma is a common malignant disease with dismal survival statistics (34-36) and one for which there are no effective therapeutic measures after the disease has advanced beyond the early stages. Spremulli and Dexter (37) have suggested the importance of examining the response of colon carcinoma cells to maturational compounds. In the present study we extend our investigations regarding cell surface response of transformed cells to DMF to a human colon cancer cell line. The HCT MOSER human colon cancer cell line was used in this research. HCT MOSER is a cell line established in vitro from a primary tumor obtained at surgery (38) and, by virtue of in vitro criteria, is a cell line that has been classified as a moderately differentiated, intermediately aggressive colon carcinoma line (24, 38). HCT MOSER has also been identified as a colon cancer line capable of elaborating TGFs that produce transformation of AKR-2B cells (32, 39, 40).

MATERIALS AND METHODS

Materials. Lactoperoxidase, glucose oxidase, anti-rabbit IgG-FITC conjugate, mithramycin A, and DNA type I from calf thymus were obtained from Sigma Chemical Co. (St. Louis, MO). Mouse fibronectin was purchased from Biomedical Technologies, Inc. (Cambridge, MA). Anti-human fibronectin and human fibronectin were from Collaborative Research (Lexington, MA). Carrier-free Na125I at a concentration of 100 mCi/ml was obtained from Amersham (Arlington Heights, IL). Rabbit anti-human CEA was a gift from DAKO Corp. (Santa Barbara, CA). All reagents and equipment for SDS-PAGE, including molecular weight standards, were purchased from Bio-Rad (Richmond, CA). ACS grade DMF was obtained from Fisher Chemical Co. (Pittsburgh, PA). Cell culture supplies were obtained from Grand Island Biological Co. (Grand Island, NY) and Flow Laboratories (McLean, VA). Cell cultures were grown in 25-cm2 Corning disposable plastic flasks (Corning, NY). 125I-labeled Protein A with a specific activity of 26.0 μCi/μg was obtained from ICN Biomedicals, Inc. (Irvine, CA).

Cell Culture. Stock cultures of the AKR-MCA cell line (kindly provided by Dr. H. L. Moses, Department of Cell Biology, Mayo Graduate School of Medicine, Rochester, MN) were stored in liquid nitrogen. Working cultures of the line were maintained at 37°C and 5% CO2 in McCoy's Tissue Culture Medium 5A supplemented with 10% heat-inactivated FBS (v/v) and gentamicin (52 μg/ml). AKR-MCA cultures were discarded after being subcultured 10 times. The HCT MOSER human colon cancer cell line was established in vitro from a spontaneously arising lesion as described previously (38). HCT MOSER cells were maintained in culture using the same conditions as those for AKR-MCA cells.

HCT MOSER cells were exposed to 0.1-0.5% DMF (v/v) in PBS-supplemented culture medium 1 day after inoculation into 25-cm2 culture flasks. Cells were grown in the presence of DMF for specified times prior to radiolabeling in situ. During the times of DMF treatment, HCT MOSER cultures were not subcultured. Fresh DMF-containing medium was applied as necessary.

AKR-MCA and HCT MOSER cells were also grown in the presence and absence of DMF on glass microscope coverslips placed in 6-well culture dishes. Cells cultured in this manner were used for cell surface immunofluorescence studies. Consistent with past experimentation (33), AKR-MCA cells were grown in culture medium containing 1.0% DMF. AKR-MCA cells were exposed to DMF 4 days before immunofluorescent labeling while HCT MOSER cells were grown in DMF-containing medium for 21 days before labeling.

Preparation of Anti-Mouse Fibronectin. Rabbits were given initial injections of 150 μg of mouse fibronectin suspended in Freund's complete adjuvant. Approximately 75% of this quantity was injected s.c. in the neck region and the remaining 25% was injected intradermally in the flank. This injection procedure was repeated 1 week later with another 150 μg of mouse fibronectin, suspended this time in Freund's incomplete adjuvant. One week after the second series of injections the rabbit received 100 μg of mouse fibronectin contained in PBS. The injection sites and the proportions of material injected into each site were the same as for the first and second injection series. After the third round of injections phlebotomy was performed using a heparinized syringe. The plasma was harvested and sodium azide was added to a final concentration of 0.1% (w/v). The plasma/sodium azide was divided into aliquots and stored at −20°C.

Cell Surface Radiolabeling. Radiodiagenation of HCT MOSER cell surface proteins was accomplished using cultured cells in situ and methods detailed previously (33). Separate cultures of HCT MOSER cells and HCT MOSER cells subjected to 0.5% DMF were radiodiagenated and harvested in PBS for DNA assay and subsequent determination of 125I incorporation per μg DNA.

Collection of Shed Cell Surface Proteins and Excreted/Secreted Proteins. Radiolabeled and nonradiolabeled HCT MOSER cell cultures were washed 3 times with FBS-free McCoy's medium. McCoy's medium was placed on the cells for 1 h at 37°C, removed and replaced with fresh McCoy's in which the cells were incubated for 23 h at 37°C. Serum-free McCoy's containing DMF was used in place of plain McCoy's for collection of shed cell surface proteins and excreted/secreted proteins from cells grown in the presence of DMF. After the 23-h incubation the serum-free medium was collected, passed through a 0.2-μm filter, dialyzed against leupeptin, 5 μg/ml, at 4°C overnight, exhaustively lyophilized, and stored at −70°C. Cell cultures were mechanically harvested in PBS for DNA assay after collection of the conditioned medium.

Lypophilized material was prepared for electrophoresis by solubilization in 2.5% SDS, 5% β-mercaptoethanol, and 3 mM phenylmethylsulfonyl fluoride at 70°C for 20 min with occasional vortexing followed by centrifugation. The supernatant was applied to SDS-PAGE.

As the lactoperoxidase-glucose oxidase radiodiagenation technique labels only the externally oriented plasma membrane proteins of viable cells (11), 125I-labeled proteins contained in the serum-free conditioned medium must have been shed from cell surfaces. All other proteins in the serum-free medium were designated excreted/secreted proteins.

Immunofluorescent Labeling. Cells grown on glass coverslips were washed with PBS at 37°C and then incubated in PBS-1% BSA at 37°C for 20 min. Three ml of fresh PBS-1% BSA, containing 50 μl of anti-human fibronectin, 20 μl of anti-human CEA, or 50 μl of anti-mouse fibronectin, was placed on the cells for 20 min at 37°C. The cells were then washed with PBS-1% BSA at 22°C followed by incubation for 20 min at 22°C in 3 ml of PBS-1% BSA in which there were 30 μl of anti-rabbit IgG-FITC conjugate. After exposure to the anti-IgG-FITC conjugate the cells were washed with PBS and viewed under a Nikon microscope equipped for fluorescence microscopy and photomicroscopy.

SDS-PAGE and Electrophoretic Transfer of Proteins to Nitrocellulose. Radiodiagenated cell surface proteins and shed cell surface proteins from HCT MOSER cells were electrophoresed on 6% acrylamide cylindrical gels and on 5-10% linear gradient acrylamide slab gels using the method of Weber and Osborn (41). Cylindrical gels on which labeled proteins were electrophoresed were sliced at 1-mm intervals after being electrophoresed. Those regions of slab gels that contained radiolabeled HCT MOSER cells were electrophoresed on slab gels as above and then transferred onto nitrocellulose sheets (Bio-Rad) according to the procedure of Towbin et al. (42) and using a Hoefer Scientific Instruments Transphor apparatus (San Francisco, CA). The nitrocellulose membranes onto which proteins were transferred were incubated in 5% BSA, 0.05% Tween-20 in PBS overnight followed by incubation...
SYNTHESIS OF ANTI-FIBRONECTIN-REACTIVE PROTEIN

overnight in 50 ml of BTP containing 100 µl of anti-human fibronectin. After washing with BTP, the nitrocellulose sheets were placed overnight in 50 ml of BTP to which ~5 x 10⁶ cpm of ¹²⁵I-labeled Protein A was added. Following exposure to labeled Protein A the sheets were washed with PBS, dried, and autoradiographed. All washings and incubations were performed at 22°C.

DNA Assay. Cellular DNA content was determined by the method of Hill and Whatley (43) using calf thymus DNA as standard.

RESULTS

Growth of HCT MOSER cells in 0.5% DMF resulted in morphological changes that occurred in a time-dependent manner, as shown in Fig. 1. HCT MOSER cells not exposed to DMF (Fig. 1A) exhibited a round shape in vitro and grew in cell clusters in which there were indistinct cell boundaries and in which many cells were not in contact with the flask. Continuous exposure of HCT MOSER cells to 0.5% DMF for 14 days yielded cells that were flattened and elongated (Fig. 1B). Exposure to DMF for an additional 7 days produced further flattening of HCT MOSER cells, complete attachment of cells to the flask, acquisition of a fibroblastic appearance, and growth in a monolayer fashion (Fig. 1C). The degree of confluence of HCT MOSER cultures treated with DMF was not a factor in DMF-induced morphological alterations. At culture confluence, HCT MOSER cells grown in DMF for 21 days manifested the same flattened, fibroblastic aspect as subconfluent cultures. HCT MOSER cells in cultures approaching confluence retained the same appearance as cells in markedly subconfluent cultures. Removal of DMF from HCT MOSER cells grown in DMF for 21 days resulted in reversion to a cellular morphology identical to that of HCT MOSER cells never exposed to DMF (Fig. 2). This morphological reversion occurred within 5 days after removal of DMF.

Cell surface changes associated with DMF treatment of HCT MOSER cells coincided with changes in morphology. Fig. 3A is representative of the electrophoretic profiles of ¹²⁵I-labeled cell surface proteins from HCT MOSER cells. These profiles are characterized by distinct radiolabeled surface protein peaks with molecular weights of 180,000 (csp180) and 120,000 (csp120). HCT MOSER cells also expressed a significant amount of surface material of approximately 87,000 molecular weight that was susceptible to labeling. Radioiodination of HCT MOSER cells grown in 0.5% DMF for 14 days revealed a decrease in csp120, a relative increase of 2 labeled protein species in the 120,000–180,000 molecular weight range, and a relative increase in csp180 (Fig. 3B). The labeling patterns of HCT MOSER cells exposed to DMF for 21 days (Fig. 3C) showed further reduction in csp120 and absence of M, 87,000 protein. Electrophoretic profiles of HCT MOSER cells treated with DMF for 21 days were dominated by a single peak representing labeled surface protein of about M, 200,000 (csp200) and resembled the labeling profiles of nontransformed IMR-90 human fibroblasts (Fig. 3D).

Radioiodinated surface protein from IMR-90 fibroblasts, HCT MOSER colon carcinoma cells, and HCT MOSER cells grown for 21 days in 0.5% DMF was electrophoresed on slab gel followed by autoradiography for direct comparison of protein migration. The most prominently labeled cell surface component from IMR-90 (Fig. 4C) and DMF-treated HCT MOSER (Fig. 4B) cells appears to be of the same molecular weight. A radioiodinated protein of corresponding electrophoretic mobility is not apparent in the lane in which material from untreated HCT MOSER cells was run (Fig. 4A).

In addition to the morphology reversion produced by removal of DMF from HCT MOSER cells, cells exposed to DMF for 21 days and then grown in the absence of DMF also exhibited a cell surface labeling pattern (Fig. 5) very similar to that of untreated HCT MOSER cells (Fig. 3A). Any differences between the Fig. 3A and Fig. 5 profiles tend to be of a quantitative, rather than qualitative, nature.

Cell surface response of HCT MOSER cells to DMF was concentration dependent. HCT MOSER cells were grown for 21 days in medium containing 0.1, 0.2, and 0.3% DMF prior to radioiodination. Incubation in 0.1% DMF produced minimal cell surface changes (Fig. 6A), but increasing the DMF concentration to 0.2% caused the appearance of a surface protein with a molecular weight greater than that of csp180 (Fig. 6B). This surface protein was further increased when HCT MOSER cells were grown in 0.3% DMF (Fig. 6C). Reduction of the csp120 and M, 87,000 proteins, a prominent feature in the surface

Fig. 1. Effect of 0.5% DMF on HCT MOSER cell morphology. HCT MOSER cells were grown in A, 25-cm² culture flasks in the absence of DMF, B, in the presence of DMF for 14 days and C, in the presence of DMF for 21 days.
SYNTHESIS OF ANTI-FIBRONECTIN-REACTIVE PROTEIN

Fig. 2. Reversal of DMF-induced morphological alterations in HCT MOSER cells exposed to DMF for 21 days and then removed from the influence of DMF for 5 days.

profiles of HCT MOSER cells subjected to 0.5% DMF, did not occur with the use of DMF concentrations ≤0.3%. However, there was a tendency for csp180 to decrease when HCT MOSER cells were exposed to 0.3% DMF.

The possibility that growth of HCT MOSER cells in DMF induced the elaboration of fibronectin was suggested by the appearance of a DMF-related surface protein susceptible to radioiodination, which comigrated with the single largest labeled surface protein from IMR-90 fibroblasts, and by the effect of DMF on HCT MOSER cell morphology. Similar morphological and cell surface events were observed previously when transformed AKR-MCA fibroblasts were grown in DMF (33). The identity of a M, 200,000–250,000 surface protein, which was increased on AKR-MCA cells post-DMF treatment and decreased on AKR-2B cells upon transformation, has been postulated to be fibronectin (33). Cell surface immunofluorescence techniques were used to explore the possibility that csp200 from DMF-treated HCT MOSER cells and the M, 200,000–250,000 surface protein of AKR-MCA cells exposed to DMF might be fibronectin.

AKR-MCA and HCT MOSER cells were incubated in anti-mouse and anti-human fibronectin, respectively, and then exposed to the appropriate anti-IgG-FITC conjugate. DMF-treated AKR-MCA and HCT MOSER cells were also exposed to anti-fibronectin and anti-IgG-FITC. Fig. 7 shows that, before growth in DMF, AKR-MCA cells expressed little surface material reactive with anti-mouse fibronectin. Growth in DMF-containing medium for 4 days resulted in induction of material on the surfaces of AKR-MCA fibroblasts that was strongly reactive with anti-mouse fibronectin and had a fibrillar pattern (Fig. 8). Similarly, prior to DMF exposure HCT MOSER cells evidenced no detectable surface fibronectin (Fig. 9), while growth in DMF-supplemented medium resulted in the presence...
SYNTHESIS OF ANTI-FIBRONECTIN-REACTIVE PROTEIN

Fig. 4. Direct comparison of the electrophoretic mobilities of $^{125}$I-labeled cell surface proteins from HCT MOSER cells (lane A), HCT MOSER cells grown in 0.5% DMF for 21 days (lane B), and normal IMR-90 fibroblasts (lane C). An equal amount of radiolabel was applied to each lane. Molecular weight standards migrated as indicated (molecular weight $\times 10^{-3}$).

Fig. 5. Reversal of the cell surface effects of DMF. Cell surface electrophoretic pattern of HCT MOSER cells grown in 0.5% DMF for 21 days and then grown in the absence of DMF for 5 days.

of a surface component immunoreactive with anti-fibronectin (Fig. 10B).

Contaminant fibroblasts in HCT MOSER cultures may have been selected for and allowed to proliferate secondary to exposure of the colon cancer cell cultures to DMF. This would account for the presence of cells with surface constituents reactive with anti-fibronectin. However, cells comprising DMF-treated HCT MOSER cultures continued to produce surface CEA as indicated by surface fluorescence after incubation in anti-human CEA and anti-IgG-FITC (Fig. 11B). This suggests that the cells comprising DMF-treated HCT MOSER cultures were of colonic origin.

HCT MOSER cells and HCT MOSER cells subjected to DMF were assayed for the amount of surface protein per cell
SYNTHESIS OF ANTI-FIBRONECTIN-REACTIVE PROTEIN

Fig. 7. A, phase-contrast photomicrograph of AKR-MCA cells incubated in antisera to mouse fibronectin prepared in rabbit followed by exposure to anti-rabbit IgG-FITC conjugate; B, fluorescence of the same cells.

Fig. 8. A, phase-contrast image of AKR-MCA cells grown in 1.0% DMF for 4 days; B, fluorescence pattern of surface fibronectin on the same cells. Immunofluorescence was performed as described in “Materials and Methods.”

Fig. 9. A, phase-contrast, and B, fluorescence images of HCT MOSER cells incubated in anti-human fibronectin followed by exposure to anti-rabbit IgG-FITC conjugate.
SYNTHESIS OF ANTI-FIBRONECTIN-REACTIVE PROTEIN

Fig. 10. Expression of surface fibronectin-like material on HCT MOSER cells treated with DMF for 21 days. A, phase-contrast, and B, fluorescence photomicrographs of cells labeled with anti-human fibronectin and anti-rabbit IgG-FITC.

Fig. 11. Demonstration of the presence of surface CEA on HCT MOSER cells exposed to DMF for 21 days. A, phase-contrast, and B, fluorescence images of cells labeled with anti-CEA followed by anti-IgG-FITC.

available for radiiodination. A measure of this was obtained by determining $^{125}$I incorporation per $\mu g$ DNA. The results of this determination are presented in Table 1. As indicated, there was relatively little change in the amount of $^{125}$I-labeled surface protein per cell before and after exposure to DMF.

It has been shown that cell surface protein of transformed cells is shed or released (33, 44, 45). Bystryn and Perlstein (45) have proposed that shedding of surface macromolecules from tumor cells is one pathway for the turnover of surface protein. In consideration of this, HCT MOSER cells were examined for differences in shed cell surface protein composition resulting from exposure to DMF. The electrophoretic fractionation of shed cell surface protein from HCT MOSER cells and HCT MOSER cells treated with DMF for 21 days is depicted in Fig. 12, A, B, and C, respectively. Although there was a progressive decrease of cspl20 on HCT MOSER cells with increased time of exposure to DMF, all 3 shed cell surface protein profiles exhibit a prominent amount of $^{125}$I-labeled material with a molecular weight of 120,000. Curiously, while HCT MOSER cells had a large amount of cspl20 (Fig. 3A), the shed cell surface protein from these cells (Fig. 12A) contained little, if any, $M_i$ 180,000 protein. Significantly, HCT MOSER cells grown in DMF shed a substantial quantity of $M_i$ ~200,000 surface protein, the amount of which was directly proportional to time of growth in DMF (Fig. 12, B and C).

To determine whether HCT MOSER cells, grown in the presence and absence of DMF, released fibronectin into culture medium, conditioned medium from these cells was collected, electrophoresed, and transferred to nitrocellulose sheets that were incubated with antisera and $^{125}$I-Protein A as described. An amount of material from an equal number of cells (based on DNA assay) was electrophoresed in each lane. There was no detectable amount of material immunoreactive with anti-fibro-

Table 1 Incorporation of $^{125}$I into HCT MOSER cell surface protein per $\mu g$ DNA

<table>
<thead>
<tr>
<th>Time of growth in 0.5% DMF at time of labeling (days)</th>
<th>$(cpm/\mu g$ DNA) $\pm$ SD</th>
<th>$\times 10^{-3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.35 $\pm$ 0.05</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1.27 $\pm$ 0.20</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>1.45 $\pm$ 0.15</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>1.48 $\pm$ 0.16</td>
<td></td>
</tr>
</tbody>
</table>

$^{125}$I activity was reference dated to correct for radioactive decay.
SYNTHESIS OF ANTI-FIBRONECTIN-REACTIVE PROTEIN

Fig. 12. A, shed cell surface proteins from HCT MOSER cells; B, HCT MOSER cells grown in 0.5% DMF for 14 days; C, HCT MOSER cells grown in 0.5% DMF for 21 days. Arrows in B and C indicate the approximate M, 200,000 shed surface protein that was increased as a function of time of exposure of HCT MOSER cells to DMF.

nectin in the medium in which HCT MOSER cells were grown (Fig. 13B). This is in marked contrast to DMF-treated HCT MOSER cells, which clearly synthesized and released into medium material reactive with anti-fibronectin (Fig. 13, C and D). The molecular weight of the immunoreactive material from DMF-treated HCT MOSER cells approximates that of cellular fibronectin, which is 220,000 ± 20,000.

To control for binding of fibronectin contained in FBS to HCT MOSER cells and its later release into serum-free medium, FBS was assayed for material reactive with anti-human fibronectin. To this end, FBS was electrophoresed and transferred onto nitrocellulose sheets in the same way as conditioned medium from DMF-treated HCT MOSER cells. The nitrocellulose sheets were then incubated under anti-human fibronectin and 125I-labeled Protein A as described in "Materials and Methods." FBS was found to contain no detectable material immunoreactive with anti-human fibronectin (data not shown). Likewise, no anti-human fibronectin-immunoreactive material was detected in whole HCT MOSER cells prior to growth in DMF. This indicates that DMF did indeed induce synthesis of anti-fibronectin-reactive material and did not merely cause the mobilization of an existing cytoplasmic pool.

Cellular viabilities, as determined by trypan blue exclusion, for HCT MOSER cultures after radioiodination, ranged between 90 and 95%. Viabilities of HCT MOSER cultures subjected to DMF treatment varied between 81 and 89%.

DISCUSSION

Application of DMF to culture medium in which the spontaneously arising human colon cancer line HCT MOSER was grown resulted in cell surface alterations coincident with cell morphology changes. The cell surface and morphology changes were reversible as well as dependent upon time of exposure to DMF and DMF concentration. DMF has been noted to reversibly produce decreased tumorigenicity and clonogenicity in human colon cancer cells (4, 37) and to reversibly modify expression of human colon cancer cell tumor-related markers (4, 5). These findings indicate that in vitro exposure of human
colon carcinoma cells to DMF induces a more benign, better differentiated phenotype. Thus, the cell surface response of HCT MOSER cells to DMF may be associated with induced differentiation.

The AKR-2B/AKR-MCA mouse fibroblast cell lines were used as a model system to initially investigate and document any cell surface manifestations related to DMF-induced differentiation (32, 33) before examining the effects of DMF on a transformed cell line for which there is no untransformed counterpart for comparison, e.g., HCT MOSER. Despite the species and cell type differences, there are parallels between the cell surface effects of DMF on HCT MOSER and the previously described actions on methylcholanthrene-transformed AKR-MCA mouse fibroblasts (33). Both cell lines exhibited a surface protein with a molecular weight of 85,000–87,000, which was decreased upon DMF treatment. The amounts of cell surface proteins with molecular weights less than 200,000 tended to be diminished when HCT MOSER and AKR-MCA cells were exposed to DMF. Other similarities between the two cell lines and their response to DMF include: synthesis of TGFs (40, 46); cell surface alterations produced by DMF as a function of time of exposure to DMF and DMF concentration; similar cell and culture morphologies following DMF treatment; and DMF-enhanced production of cell surface-associated material immunoreactive with anti-fibronectin.

The possibility that the anti-fibronectin-immunoreactive material on the surfaces of DMF-treated AKR-MCA and HCT MOSER cells and released from DMF-treated HCT MOSER cells actually is fibronectin is potentially important. Fibronectin is related to cellular differentiation (11, 47–49), control of cell replication (50), transformation (51), regulation of gene expression (52), and modulation of invasiveness and metastasis of malignant cells (53, 54). The effect of DMF on HCT MOSER and AKR-MCA cell morphology is consistent with expression of fibronectin as fibronectin promotes cell attachment and spreading (see Refs. 48, 49, 53, 55 for reviews). Furthermore, the anti-fibronectin-reactive material on the surfaces of DMF-treated AKR-MCA cells exhibited a fibrillar arrangement compatible with the appearance of fibronectin matrix on nontransformed cells (49, 53, 55). This may be regarded as supporting the contention that the DMF-induced surface material on AKR-MCA cells reactive with anti-fibronectin is indeed fibronectin. Expression of fibronectin by AKR-MCA cells after exposure to a differentiation-inducing agent is certainly expected since the AKR-MCA cell line originated from nontransformed fibroblasts, which normally manifest surface fibronectin (see Refs. 11, 48, 55 for reviews). Thus, the induced presence of an anti-fibronectin-immunoreactive surface material on AKR-MCA cells correlates with the maturational properties of DMF. Low-level synthesis of fibronectin by intestinal epithelial cells in vitro has been reported (see Ref. 48 for review). Therefore, as with AKR-MCA cells, DMF-enhanced synthesis of fibronectin by HCT MOSER would be anticipated if a more differentiated phenotype was assumed. Two observations indicate that fibroblast contamination was not responsible for production of anti-fibronectin-immunoreactive material by DMF-treated HCT MOSER cultures: (a) there was continued elaboration of CEA by HCT MOSER cells after exposure to DMF; and (b) removal of DMF from HCT MOSER cells resulted in prompt reversion to a pre-DMF phenotype. Hence, those cells of DMF-treated HCT MOSER cultures apparently possessed certain characteristics of colon cancer cells that should not be displayed by fibroblasts.

Unlike AKR-MCA cells subjected to DMF, the pattern of material reactive with anti-fibronectin on the surfaces of DMF-treated HCT MOSER cells was diffuse rather than fibrillar. Also, 125I incorporation data imply that there was no buildup over time of the surface protein with a molecular weight of approximately 200,000 on HCT MOSER cells secondary to DMF treatment as was shown previously for DMF-treated AKR-MCA cells (33). However, csp200 was essentially the only surface protein available for radioiodination on HCT MOSER cells grown in DMF for 21 days. If csp200 is the same as anti-fibronectin-immunoreactive material, then this surface component may be only transiently associated with the cell surface before being released from the cell. Inability to retain the anti-fibronectin material on the surface may be related to the lack of a well-defined fibrillar pattern on DMF-treated HCT MOSER cells.

If the DMF-induced anti-fibronectin-immunoreactive material produced by HCT MOSER cells is fibronectin, then possible reasons for its failure to accumulate on the cell surface include the following: absence of the normal complement of matrix components (48, 49, 53); alteration of the carbohydrate moieties of cell surface glycoconjugates (49, 56); and modification of the fibronectin molecule via phosphorylation/dephosphorylation or glycosylation processes (57, 58). It has been shown that retinoic acid (a differentiation agent) can affect the glycosylation of fibronectin (56, 59). However, the carbohydrate moieties of fibronectin are not involved in the biological functions of fibronectin (48).

It was observed during this study that trypsin treatment of HCT MOSER cells grown in DMF for 14–21 days caused the cells to exhibit a pre-DMF morphology and cell surface profile. This suggests that the morphological effects of DMF on HCT MOSER cells were mediated by a cell surface-associated protein that required a relatively long amount of time (2–3 weeks) to accumulate and to manifest its actions. DMF-induced fibronectin could produce the phenotypic response of HCT MOSER cells to DMF via interaction with the extracellular matrix as the extracellular matrix functions in the regulation of gene expression and differentiation (60–64). In this scenario, cells capable of producing fibronectin could promote differentiation in cells that are unable to synthesize fibronectin but are proximate to the cells that do.

We have suggested previously that AKR-2B/AKR-MCA surface proteins, of molecular weight less than 200,000, which are increased upon transformation and eliminated upon DMF treatment, may be fibronectin fragments (33, 39). Such a proposal is consistent with observations and proposals made by others that relate to proteolytic degradation of fibronectin in transformed cells (11, 48, 65, 66). This postulate is significant because certain proteases have been shown to be associated with transformation (65, 67) and fibronectin fragments have been shown to enhance transformation (51). However, if the csp85 and csp63 from AKR-MCA cells and the csp180 and csp120 from HCT MOSER cells are fibronectin fragments, they lack an epitope reactive with the antisera to fibronectin used in this study.

The above discussion has concentrated on the possible role of fibronectin in DMF-induced differentiation. However, production of anti-fibronectin-immunoreactive material by DMF-treated HCT MOSER cells may simply be a manifestation of differentiation and have no function in the process. Bradshaw and Sporn (68) and Sporn et al. (69) have postulated that the differentiation-induction mechanisms of transformed cells may involve TGFs. As TGFs have been identified in the conditioned medium and cell extracts of the HCT MOSER line (39, 40),
REFERENCES


N,N-Dimethylformamide-induced Synthesis of an Anti-Fibronectin Reactive Protein in Cultured Human Colon Carcinoma Cells

Michael E. Marks, Barry L. Ziober and Michael G. Brattain


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/10/5248

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.