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Differential Expression of a Tropomyosin Isoform in Low- and High-Metastatic Lewis Lung Carcinoma Cells

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Two-dimensional electrophoretograms of newly synthesized polypeptides from low-metastatic (P29) and high-metastatic (D6) Lewis lung carcinoma cells were compared. The results showed that the synthesis of tropomyosin 2 (TM2) was significantly less in D6 cells than in P29 cells. Furthermore, suppression of TM2 synthesis was induced in P29 cells during incubation in medium containing dimethyl sulfoxide or butyric acid, which induced the metastatic phenotype of P29 cells. These results suggest that the suppression of TM2 synthesis is linked to the metastatic potential of Lewis lung carcinoma cells.

Cultured mammalian cells contain multiple forms of tropomyosin (TM) with a broad range of molecular weights (8, 13, 15, 17, 23). These nonmuscle TMs are associated with actin in microfilaments (10, 17). Their function in these filaments is not clear, but it has been suggested that they stabilize the actin filament network through the inhibition of the action of gelsolin or the actin-depolymerizing factor (2, 6). Recently, the syntheses of major isoforms of TM have been reported to be suppressed in cells that are transformed by retroviruses (8, 9, 15), as well as in cells that are transformed by viral oncogenes (4), chemical carcinogens and UV irradiation (11, 13), and naturally occurring tumor cell lines (13, 14). These changes in the pattern of TM synthesis may be responsible, in part, for the derangement of microfilament bundles to a more dispersed, less orderly state (12), resulting in the changes of cell shape and motility that are considered to be characteristics of transformed and more malignant cells. To date, however, it is unknown whether the change in the pattern of TM synthesis is responsible for or involved in the progression of malignancy, especially in the acquisition of the metastatic phenotype of tumor cells. In this study we compared two-dimensional electrophoretograms of newly synthesized polypeptides from low-metastatic (P29) and high-metastatic (D6) Lewis lung carcinoma cells. We found that the synthesis of a major TM was significantly less in D6 cells than in P29 cells and that its synthesis in P29 cells was inhibited by dimethyl sulfoxide (DMSO) or butyric acid, both of which induced a high-metastatic phenotype in P29 cells.

Cloned low-metastatic Lewis lung carcinoma P29 cells and high-metastatic D6 cells were used in this study. The origin of the carcinoma and the isolation and characteristics of P29 cells have been described previously (22). D6 cells were isolated by the method described previously (22), except that in vivo-in vivo selection was repeated 60 times. The metastatic abilities of P29 and D6 cells are shown in Table 1. When P29 and D6 cells were injected intravenously (experimental metastasis), P29 cells formed only a few metastatic pulmonary nodules, whereas D6 cells formed many. When these cells were injected intramuscularly (spontaneous metastasis), similar results were obtained. When P29 cells were treated with 280 mM DMSO or 1 mM butyric acid for 5 days and then injected intravenously into syngeneic mice, they formed many metastatic pulmonary nodules (Table 1) (22).

Newly synthesized polypeptides in P29, D6, and P29 cells treated with DMSO or butyric acid were analyzed by two-dimensional polyacrylamide gel electrophoresis (Fig. 1). For this, subconfluent monolayers of cells were labeled for 2 h with 50 μCi of [^{35}S]methionine (1,120 Ci/mmol; Dupont, NEN Research Products, Boston, Mass.) per ml in methionine-free Dulbecco modified Eagle medium containing 10% dialyzed fetal bovine serum. Whole-cell extract was prepared by lysing cells directly in lysis buffer (19), and cellular polypeptides were analyzed by the method of O'Farrell (19). Comparison of the autoradiograms showed lower labeling of a polypeptide (M_r = 36,000, pI = 4.5; indicated by number 2 in Fig. 1B through E) in D6 and P29 cells treated with DMSO or butyric acid than in P29 cells (Fig. 1B through E). We identified this polypeptide as a TM isoform, TM2, on the basis of (i) its molecular weight and pI value, (ii) its cross-reactivity with rabbit antiserum to chicken gizzard TM, (iii) its heat stability, and (iv) the absence of proline and tryptophan in this polypeptide (16, 20) (data not shown). In addition to TM2, at least four other TM isoforms, TM1, TM3, TM4, and TM5, were identified on the basis of the same criteria (Fig. 1), which are consistent with the results of previous reports (13, 15, 17).

For quantitation of the syntheses of TMs in P29, D6, and P29 cells treated with DMSO or butyric acid, the spots corresponding to TMs and actin were cut out of the gel and their radioactivities were counted. The ratios of TM2 to actin in D6 cells and P29 cells treated with DMSO or butyric acid decreased significantly (Table 2). However, there was no significant difference in the ratios of other TMs to actin among these cell lines, except that there was some reduction in the ratio of TM3 to actin in butyric acid-treated P29 cells (Table 2). The smaller amount of TM2 in D6 cells and P29 cells treated with DMSO or butyric acid than in P29 cells was also demonstrated by Coomassie blue staining of the gel, indicating a lower steady-state amount of TM2 (data not shown). We next analyzed the polypeptides in Triton X-100-insoluble (cytoskeletal and nuclear) fractions (1) of P29, D6, and P29 cells treated with DMSO or butyric acid (Fig. 2A and B). A lower labeling intensity of TM2 in D6 and P29 cells treated with DMSO or butyric acid than in P29 cells was also apparent. Thus, it is conceivable that the suppression of TM2 synthesis is linked to the metastatic potential.

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TABLE 1. Metastatic abilities of untreated P29 cells, D6 cells, and P29 cells treated with DMSO or butyric acid

Cell	No. of lung nodules/mouse by the following routes of injection[a]:	
	i.v.[b]	i.m.[c]
P29	0 ± 0	2.0 ± 1.9
D6	88.6 ± 28.6	24.1 ± 12.4
DMSO-treated P29[d]	53.9 ± 19.5	NT[e]
Butyric acid-treated P29[f]	55.3 ± 10.5	NT

[a] Values are means ± standard deviations.
[b] Cells were injected intravenously (i.v.) into C57BL/6 mice (seven mice per group) at an inoculum of 8×10^4 cells per mouse. The mice were killed 17 days later for examination of pulmonary metastases.
[c] Cells were injected intramuscularly (i.m.) into C57BL/6 mice (10 mice per group) at an inoculum of 2.5×10^5 cells per mouse. The mice were killed 27 days later for examination of pulmonary metastases.
[d] P29 cells were treated with 280 mM DMSO for 5 days.
[e] NT, Not tested.
[f] P29 cells were treated with 1 mM butyric acid for 5 days.

TABLE 2. Ratio of radioactivities of [^{35}S]methionine incorporated into TM isoforms to total actins in whole cells

TM	Ratio of TM isoforms to actin in the following cells[a]:			
	P29	D6	DMSO-treated P29[b]	Butyric acid-treated P29[c]
TM1	1.9 ± 0.3	1.8 ± 0.5	2.4 ± 0.5	2.1 ± 0.3
TM2	15.2 ± 1.7	4.5 ± 0.9[d]	5.2 ± 3.0[d]	7.0 ± 1.2[d]
TM3	4.1 ± 0.2	5.0 ± 0.8	3.8 ± 1.2	2.5 ± 0.9[e]
TM4	3.0 ± 0.4	3.2 ± 0.4	2.7 ± 0.3	2.4 ± 0.3
TM5	6.0 ± 0.7	5.6 ± 0.2	5.8 ± 0.5	5.7 ± 0.6

[a] Ratios are expressed per 100 parts of actin. Values are means ± standard deviations of triplicate determinations.
[b] P29 cells were treated with 280 mM DMSO for 5 days.
[c] P29 cells were treated with 1 mM butyric acid for 5 days.
[d] Significant difference from the value for untreated P29 cells at $P < 0.001$, as determined by Student's t test.
[e] Significant difference from value for untreated P29 cells at $P < 0.05$, as determined by Student's t test.

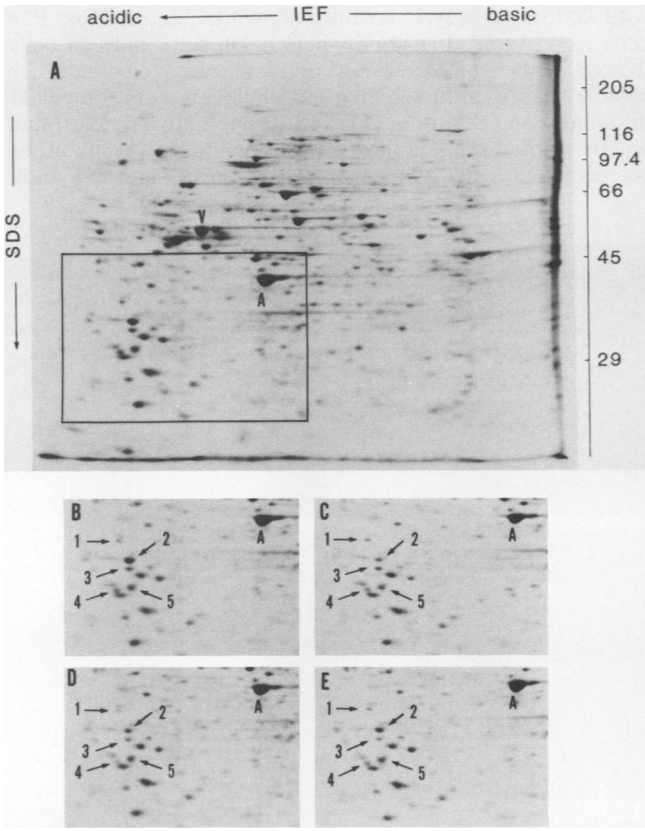

FIG. 1. Newly synthesized polypeptides of Lewis lung carcinoma cells analyzed by two-dimensional polyacrylamide gel electrophoresis. Cells were labeled with [^{35}S]methionine for 2 h. Whole-cell polypeptides were prepared, separated by two-dimensional polyacrylamide gel electrophoresis, and located by autoradiography. (A) Whole-cell polypeptides of P29 cells. Numbers on the right are apparent molecular masses, in kilodaltons. The area outlined contains polypeptides of special interest that are shown in panels B through E. IEF, Isoelectrofocusing; SDS, sodium dodecyl sulfate. (B through E) Polypeptides of P29 cells, D6 cells, P29 cells treated with 280 mM DMSO for 5 days, and P29 cells treated with 1 mM butyric acid for 5 days, respectively. Numbers 1 through 5 indicate the locations of TM1, TM2, TM3, TM4, and TM5, respectively. Actin (A) and vimentin (V) are also indicated.

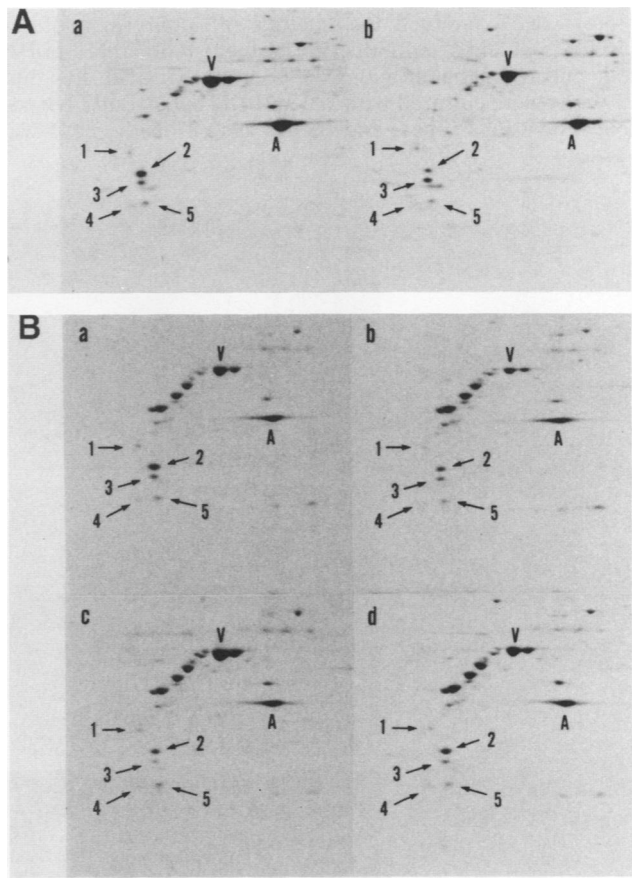

FIG. 2. Polypeptides in Triton X-100-insoluble fractions of various cells. (A) Polypeptides of P29 (a) and D6 (b) cells. (B) Polypeptides of untreated P29 cells (a) and P29 cells treated with 280 mM DMSO (b), 1 mM butyric acid (c), or 0.5 mM 8-bromo-cyclic AMP (d) for 5 days. Cells were labeled with [^{35}S]methionine for 2 h. Triton X-100-insoluble polypeptides were prepared and analyzed by two-dimensional polyacrylamide gel electrophoresis and autoradiography. Numbers 1 through 5 indicate the locations of TM1, TM2, TM3, TM4, and TM5, respectively. Actin (A) and vimentin (V) are also indicated.

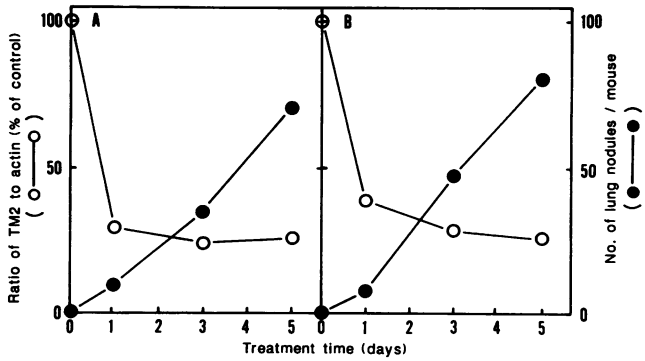

FIG. 3. Effects of DMSO and butyric acid on TM2 synthesis and metastatic ability of P29 cells. P29 cells were cultured with 280 mM DMSO (A) or 1 mM butyric acid (B). Then, samples of the cells was injected intravenously into syngeneic mice and other samples were labeled with [^{35}S]methionine for 2 h. Whole-cell polypeptides were separated by two-dimensional polyacrylamide gel electrophoresis. The spots corresponding to TM2 and actin were cut out, their radioactivities were measured, and the ratio of TM2 to actin was calculated.

Next, we examined the kinetics of suppression of the synthesis of TM2 and induction of metastatic ability in P29 cells during treatment with DMSO or butyric acid. For this, P29 cells were cultured with 280 mM DMSO or 1 mM butyric acid for various times, and then a sample of the cells was injected intravenously into syngeneic mice, while another sample was labeled with [^{35}S]methionine. Whole-cell polypeptides were analyzed by two-dimensional polyacrylamide gel electrophoresis, and the results are shown in Fig. 3. On treatment with DMSO, suppression of synthesis of TM2 nearly reached a steady level within 24 h, whereas the metastatic ability increased gradually with treatment time (Fig. 3A). Similar results were obtained on treatment with butyric acid (Fig. 3B). These results suggest that the suppression of TM2 synthesis may be a prerequisite, but not sufficient alone, for the expression of metastatic ability. Metastasis consists of multiple steps and requires many properties of tumor cells, including adhesiveness and enzymes that degrade the basement membrane, such as cathepsin B, plasminogen activator, and type IV collgenase (7, 18). Therefore, suppression of TM2 synthesis followed by the expression of such properties may be necessary for completion of the metastatic process. In fact, on treatment of P29 cells with DMSO or butyric acid, some of these properties are enhanced, with a concomitant increase in the metastatic ability of the cells (22).

The morphologies of P29 and D6 cells and P29 cells treated with DMSO or butyric acid are shown in Fig. 4. Most P29 cells were round (Fig. 4A), whereas D6 cells and P29 cells treated with DMSO or butyric acid were elongated or spindle-shaped, although their morphologies were somewhat different than each other (Fig. 4B through D). To determine whether suppression of the synthesis of TM2 is a result of, or is correlated with a change in, cellular shape, we took

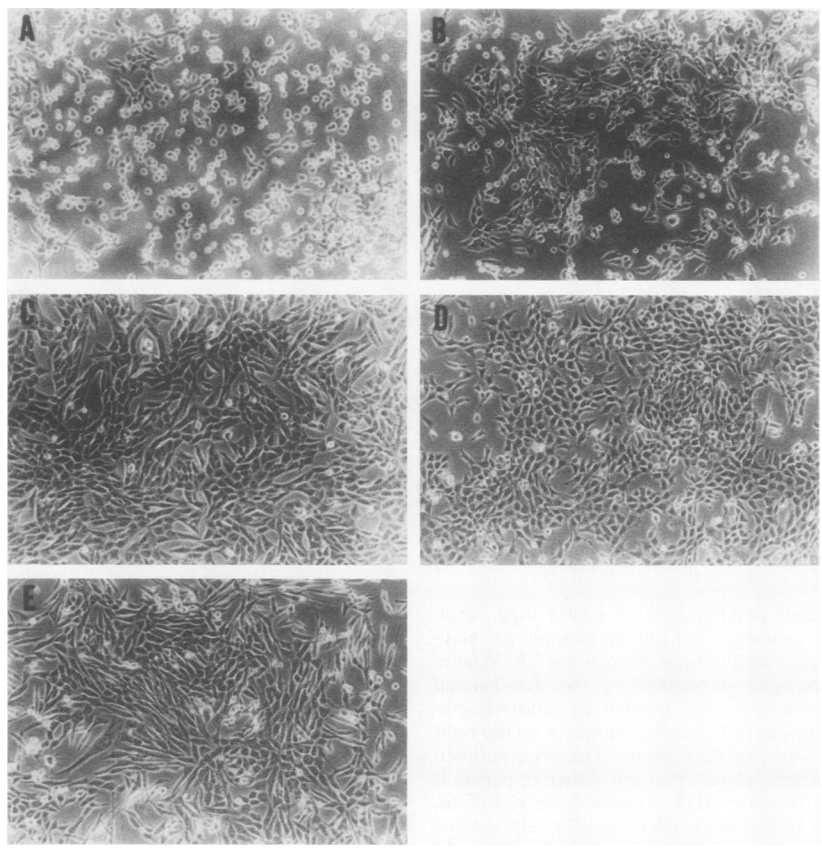

FIG. 4. Morphologies of untreated P29 cells, D6 cells, and P29 cells treated with various drugs. (A) P29 cells; (B) D6 cells; (C) P29 cells treated with 280 mM DMSO for 5 days; (D) P29 cells treated with 1 mM butyric acid for 5 days; (E) P29 cells treated with 0.5 mM 8-bromo-cyclic AMP for 5 days.

advantage of the fact that 8-bromo-cyclic AMP induces a morphological change of P29 cells to more elongated or spindle-shaped cells (Fig. 4E), but it does not induce their metastatic activity (21). This treatment did not suppress the synthesis of TM2 (Fig. 2B), suggesting that there is not necessarily any causal relationship between the change in cellular shape and the reduction in the synthesis of TM2.

The mechanism(s) by which the synthesis of TM2 is suppressed in D6 cells and P29 cells treated with DMSO or butyric acid remains to be examined. Cooper et al. (4) have observed the suppression of TM synthesis in NIH 3T3 cells transformed by the oncogenes of the *ras* family and by the kinase oncogenes, while Egan et al. (5) have reported that these oncogenes induce the metastatic phenotype in NIH 3T3 cells. Furthermore, Cooper er al. (3) have reported that transforming growth factor α suppresses TM synthesis in mouse and rat fibroblasts. From these observations, it is possible that activation of one or some of these oncogenes or production of transforming growth factor α is more pronounced in D6 cells and P29 cells treated with DMSO or butyric acid than it is in untreated P29 cells.

We do not yet know what cellular phenotypes can be altered as a consequence of TM2 suppression. Suppression of TMs with higher apparent molecular weights, which show a stronger affinity to actin than do TMs with lower molecular weights (16), is considered to result in the disorganization of actin filaments (13). This may lead cells to become round and have a change in motility. However, these morphological changes are probably not correlated with the suppression of TM2 synthesis. A plausible event may be a change in cell motility or deformability. High-metastatic tumor cells are suggested to be more motile than low-metastatic cells (7). Moreover, cell deformability may also be an important factor in the metastasis process (18). Further studies on these phenotypes may provide information about not only the functions of TMs in nonmuscle cells but also the mechanisms that are involved in metastasis.

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