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n-Butyrate Increases c-erb A Oncogene Expression in Human Colon Fibroblasts

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SUMMARY: We investigated the effect of n-butyrate (2.5 mM) on expression of the c-erb A oncogene in human colon fibroblasts. Exposure of these cells to the compound for 48 hours resulted in an increased accumulation of approximately 2.0 kb RNA which hybridized to a v-erb A cDNA probe. Hybridization to a β-actin gene probe was unaffected by the treatment. This report is the first demonstration of a factor affecting expression of the c-erb A oncogene.

Recent demonstration that the c-erb A oncogene product may be a nuclear thyroid hormone receptor (1,2) could provide important insight into the mechanism of action for 3,5,3'-l-triiodothyronine (T_3). Evidence for the functional role of the receptor in the transduction of the hormone signal is largely circumstantial (3). Identification of factors regulating its synthesis offers the potential for testing the hypothesis that binding to the product of the c-erb A oncogene is a necessary step in the mediation of T_3 regulation of transcription.

n-Butyrate, a four carbon aliphatic carboxylic acid, can modify gene expression through effects on DNA methylation, protein phosphorylation, ADP ribosylation and the acetylation of both histone and non-histone proteins (4-8). Cultured human fibroblasts respond to T_3 (9-12) and n-butyrate increases the abundance of nuclear T_3 receptors in these cells (13). This effect has been correlated with changes in the electrophoretic mobilities of histones H3 and H4, which are attributed to hyperacetylation (13).

This communication describes the results of studies designed to assess whether n-butyrate can influence the expression of c-erb A in human colon fibroblasts. We report that the abundance of hybridizable RNA for that oncogene is increased by a concentration of n-butyrate which is known to induce the T_3 receptor.

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MATERIALS AND METHODS

Cell culture and preparation. Normal human colon fibroblasts were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown to confluence in medium 199 (Whittaker, M.A. Bioproducts, Walkersville, MD) containing 10% fetal bovine serum, glutamine, penicillin and streptomycin. At confluence, some cultures were shifted to the same medium containing n-butyrate (2.5 mM), (Sigma, St. Louis, MO). Control cultures received identical medium without the compound. Following an incubation period of 48 hours, cultures were washed and RNA was prepared from cell monolayers with guanidinium chloride treatment followed by extraction in buffered, redistilled phenol and ethanol precipitation (14).

Northern blot analysis. Total cellular RNA (60 μg/lane) was electrophoresed on 1% agarose/1% formaldehyde gels (15). RNAs of known sizes (RNA Ladder, Bethesda Research Laboratories, Gaithersburg, MD) were co-electrophoresed and used as size standards. The gels were stained subsequently with ethidium bromide to document equivalent quantities of RNA in all lanes. The RNA was transferred to nylon membranes (Magnagraph, Micron Separations Inc., Honeoye Falls, NY) using standard techniques (15). The v-erb A cDNA probe (Oncor, Gaithersburg, MD), a 478 base pair fragment isolated from a region of the avian erythroblastosis virus genome containing only the v-erb A gene (16), was oligolabeled with [α-^{32}P] dCTP (Oligolabeling Kit, Pharmacia, Piscataway, NJ) (17) and allowed to hybridize to the membrane bound RNA (42^{o}C) for 16-24 hours. Membranes were then washed at high stringency and exposed to X-OMAT film (Kodak, Rochester, NY) at -70^{o}C for 3-5 days. Membranes were then stripped of radioactivity and the RNA was re-hybridized to a plasmid bearing the 3' untranslated region of human skeletal β-actin (18) labeled with [α-^{32}P] dNTP by nick-translation (15).

RESULTS AND DISCUSSION

As Figure 1 demonstrates, hybridization of the v-erb A oncogene probe to total cellular RNA from colon fibroblasts is confined to a discrete band of 2.0 kb, consistent with that reported by other workers (2). Treatment of cultures with n-butyrate (2.5 mM) for 48 hours resulted in a marked enhancement of that band. In contrast, hybridization of the same RNA preparation to the β-actin cDNA probe (Figure 2) was unaffected by n-butyrate treatment. Previous studies in our laboratory have demonstrated that this carboxylic acid fails to influence the protein content of the cell cultures or total protein synthesis (19).

These results suggest that n-butyrate increases the expression of the c-erb A gene in human colon fibroblasts. This effect occured after cultures were treated with the compound for 48 hours at a concentration known to increase the abundance of nuclear T_3 receptors in human fibroblasts (13). While hybridization to v-erb A cDNA was markedly enhanced by treatment with n-butyrate, hybridization to β-actin cDNA was uninfluenced. Figure 2 demonstrates equivalent signals in the two lanes probed with β-actin cDNA, indicating that expression of this gene was not increased by n-butyrate. This suggests that the treatment does not have a generalized stimulatory effect on transcriptional events in these cells.

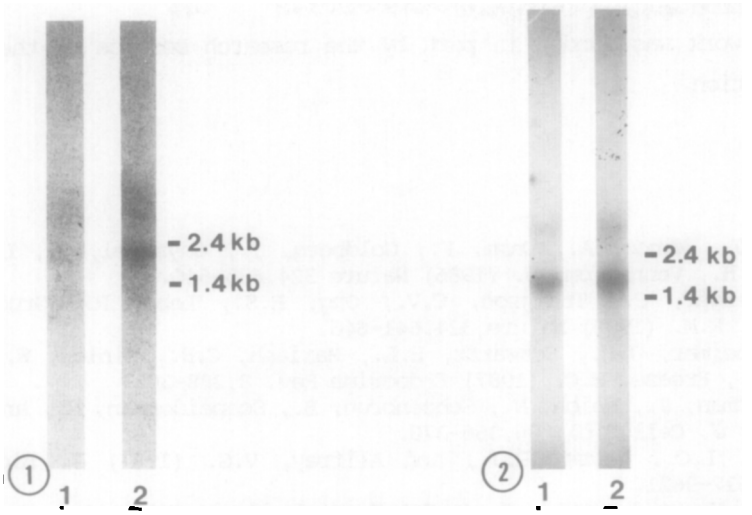

Figure 1. Northern blot analysis of human colon fibroblast RNA hybridized to [^{32}P]-radiolabeled v-erb A cDNA. Confluent cultures were incubated in the absence (Lane 1) or presence (Lane 2) of n-butyrate (2.5 mM). RNA size markers are indicated.

Figure 2. Northern blot analysis of human colon fibroblast RNA hybridized to [^{32}P]-radiolabeled β -actin cDNA. Confluent cultures were incubated in the absence (Lane 1) or presence (Lane 2) of n-butyrate (2.5 mM). RNA size markers are indicated.

Tissues investing the human colon are exposed to carboxylic acid concentrations of approximately 200 mM (20). These organic acids are generated by carbohydrate fermentation of dietary plant cell walls (21). Thus, the apparent induction of an oncogene in human colon fibroblasts by n-butyrate may represent a physiologically relevant control mechanism.

The avian erythroblastosis virus transforms erythroblasts and fibroblasts *in vitro* and induces sarcomas and erythroblastosis in birds (22). v-erb A and v-erb B are the two host cell-derived genes contained in the viral genome. The v-erb B gene confers the transforming capacity of the virus (23), and its protein product corresponds to a truncated form of the epidermal growth factor receptor (24). v-erb A, in contrast, has no independent capacity to transform primary cells in culture, but enhances the oncogenic effects of v-erb B (23). Recent reports indicate that the protein product of the c-erb A gene is a high affinity nuclear receptor for T_3. Evidence for this includes similar molecular weights and binding affinities for T_3 and its analogs (1,2). In addition, antibodies directed against the c-erb A product block T_3 binding to authentic receptor protein (1). If the protein product of the c-erb A gene is the nuclear T_3 receptor, an induction of the receptor by n-butyrate, as has been reported (13), should be accompanied by an increased expression of the c-erb A proto-oncogene. Our studies demonstrate this to be the case. Whether this induction is related to changes in the fibroblast response to T_3 is currently being examined.

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REFERENCES

1. Sap, J., Munoz, A., Damm, K., Goldberg, Y., Ghysdael, J., Lentz, A., Beng, H., Vennstrom, B. (1986) Nature 324,635-640.
2. Weinberger, C., Thompson, C.V., Ong, E.S., Lebo, R., Gruol, D.J., Evans, R.M. (1986) Nature 324,641-646.
3. Oppenheimer, J.H., Schwartz, H.L., Mariash, C.N., Kinlow, W.B., Wong, W.C.W., Frease, H.C. (1987) Endocrine Rev. 8,288-303.
4. Christman, J., Weich, N., Schoenbrun, B., Schneiderman, N., and Acs, G. (1980) J. Cell Biol. 86,366-370.
5. Boffa, L.C., Gruss, R.J., and Allfrey, V.G. (1981) J. Biol. Chem. 256,9612-9621.
6. Rastl, E., and Swetly, P. (1978) J. Biol. Chem. 253,4333-4340.
7. Kruh, J. (1982) Molec. Cell. Biochem. 42,65-82.
8. Cousens, L.S., Gallwitz, D., and Alberts, B.M. (1979) J. Biol. Chem. 254,1716-1723.
9. Voeglaar, J.P., and Erlichman, E. (1936) Am. J. Cancer 26,358-367.
10. Yoshizato, K., Kikuyama, S. and Shioya, W. (1980) Biochim. Biophys. Acta 629,23-29.
11. Chait, A., Bierman, E.L. and Albers, J.J. (1979) J. Clin. Endocrinol. Metab. 48,887-889.
12. Smith, T.J. (1984) J. Clin. Invest. 74,2157-2163.
13. Mitsuhashi, T., Uchimura, H., Takaku, F. (1987) J. Biol. Chem. 262,3993-3999.
14. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J., Rutter, W.J. (1979) Biochemistry 18,5294-5299.
15. Maniatis, T., Fritsch, E.F., Sambrook, J., eds. (1982) In: Molecular Cloning. A Laboratory Manual, pp. 149-172. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
16. Vennstrom, B., Fanshier, L., Moscovici, C., Bishop, M. (1980) J. Virol. 36,575-585.
17. Feinberg, A., Vogelstein, B. (1983) Anal. Biochem. 132,6-13.
18. Gunning, P., Ponte, P., Blau, H., Kedes, L. (1983) Molec. Cell. Biol. 3,1985-1995.
19. Smith, T.J. (1987) J. Clin. Invest. 79,1493-1497.
20. McNeil, N.I., Cummings, J.H., James, W.P.T. (1978) Gut 19,819-822.
21. Cummings, J.H. (1981) Gut 22,763-779.
22. Graf, T., Beug, H. (1978) Biochim. Biophys. Acta 516,269-299.
23. Frykberg, L., Palmieri, S., et al. (1983) Cell 32,227-238.
24. Jansson, M., Philipson, L., Vennstrom, B. (1983) EMBO J. 2,561-565.