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STIMULATION OF ANCHORAGE INDEPENDENT PROLIFERATION OF HUMAN ADRENOCORTICAL CARCINOMA CELLS BY INHIBITION OF CHOLESTEROL BIOSYNTHESIS

Damien J. Dunnington, William Prichett and Russell Greig

Department of Cell Sciences
Smith Kline & French Laboratories
King of Prussia, PA 19406-2799

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Summary: Proliferation of SW13 human adrenocortical carcinoma cells under anchorage independent conditions was stimulated in a dose-dependent manner by treatment with the cholesterol biosynthesis inhibitor mevinolin. Induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity was observed in mevinolin treated cultures. The growth stimulatory effect of mevinolin, but not that of epithelial transforming growth factor, a polypeptide growth factor for SW13 cells, was reversed by exogenous mevalonic acid. However, neither dolichol nor low density lipoprotein supplementation affected the response of SW13 cells to mevinolin. The results suggest that mevalonic acid metabolites may participate in the regulation of anchorage independent growth of SW13 cells.

Previous experiments with inhibitors of cholesterol biosynthesis have indicated that metabolites of mevalonic acid may be required for growth of a number of cell types (1,2,3). In most instances, inhibition of cholesterol biosynthesis causes growth inhibition (2). For example, Larsson & Johansson (3) showed that treatment of SV40-transformed 3T3 cultures with 25-hydroxycholesterol leads to arrest of the cells in the G_1 phase of the cell cycle. The drug-induced block could be overcome by exogenous dolichol, suggesting a role for this metabolic pathway in the maintenance of a proliferative state.

Since growth inhibition could result from deprivation of metabolites essential to normal cell function, it is difficult to distinguish between sublethal toxic effects of an inhibitor and physiological growth

The abbreviations used are HMG CoA reductase: 3-hydroxy-3-methyl glutaryl coenzyme A reductase (EC 1.1.1.34), TGFe: epithelial transforming growth factor.

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regulation. In this regard, a stimulatory response to drug treatment would be of greater interest. Studies by Nemecek, et al. (4) suggested that terbinafine, an inhibitor of squalene epoxidase, has a biphasic effect on proliferation of human fibroblasts and smooth muscle cells. Low doses of this compound stimulated thymidine incorporation by quiescent cells, whereas higher doses caused marked inhibition. In the present study, we report that anchorage independent growth of SW13 human adrenocortical carcinoma cells is stimulated by mevinolin, a potent inhibitor of HMG CoA reductase , the rate limiting enzyme in cholesterol biosynthesis.

METHODS AND MATERIALS

Assay for Anchorage Independent Growth

The soft agar assay for anchorage independent growth of SW13 cells (5) was modified to allow easy harvesting of cells. Base layers of 1 ml 1% agarose in Dulbecco's modified Eagles medium supplemented with 10% fetal bovine serum were plated in 35 mm dishes and allowed to solidify. A suspension of 5000 SW13 cells in 1 ml medium was added to each dish and test compounds were added directly to the cultures in a volume not exceeding 50 µl. After 5 days in culture, colonies were enumerated by image analysis (6).

Cell Cultures

The SW13 cell line was obtained from the American Type Culture Collection and maintained in Dulbecco's modified Eagles Medium supplemented with 10% fetal bovine serum. No mycoplasma contamination was detected by Gen-Probe assay (San Diego, CA) or by broth culture (Microbiological Associates Bethesda MD). Cells were used for experiments within 10-20 passages of receipt.

Assay of HMG CoA Reductase Activity

Cells were cultured in 10 cm petri dishes, each seeded with 10^6 SW13 cells in 5 ml medium over a base layer of 5 ml 1% agarose. Test compounds were added to the cell suspension and after 5 days, the cell layer was removed and centrifuged at 200 xg for 5 min. The pellet was trypsinized for 5 min to break up aggregates and an aliquot was removed for counting. The remainder was resuspended in medium to neutralize the trypsin, centrifuged as above and washed with 5 ml phosphate-buffered saline before being frozen at -70° for enzyme assay. Cell pellets were thawed directly into lysis buffer (50 mM KH_2PO_4, 0.2 M KCl, 5 mM EDTA, 0.25% Brij 96, pH 7.4) and the activity of HMG CoA reductase in the lysates was measured as described previously (7).

Materials

The following reagents were obtained from Sigma:mevinolin, dolichol (porcine liver) mevalonic acid lactone, human low density lipoprotein.

Epithelial transforming growth factor (TGFe) was partially purified from bovine kidney as described (6). A stock solution of 25 mM mevinolin was prepared in ethanol and diluted to 2.5 mM with 0.2 M sodium hydroxide 1 hr. before use to hydrolyse any cyclized material. Similarly, mevalonic acid lactone was prepared as a 0.25 M solution in 0.25 M sodium hydroxide. A stock solution of dolichol (1 mg/ml in ethanol) was freshly prepared for each experiment. Agarose was supplied by FMC Corporation, Rockland, ME.

RESULTS

Mevinolin induced a dose-dependent stimulation of colony formation by SW13 cells grown in suspension (Figs. 1,2). Bovine TGFe, a polypeptide growth factor for SW13 cells (8) also stimulated proliferation of the cells under the same conditions (Fig. 3). The maximum response to mevinolin was only 50% of that obtained with TGFe but was nevertheless 10 fold above background. Stimulation of colony formation was observed at concentrations of 0.25-2.5μM mevinolin and this reflected an increase in cell number (Fig. 4). At doses greater than 12.5 μM mevinolin a decline in colony formation was seen (Fig. 1).

The stimulatory effect of mevinolin on SW13 cells was undiminished in the presence of either 15 μg/ml human low density lipoprotein or

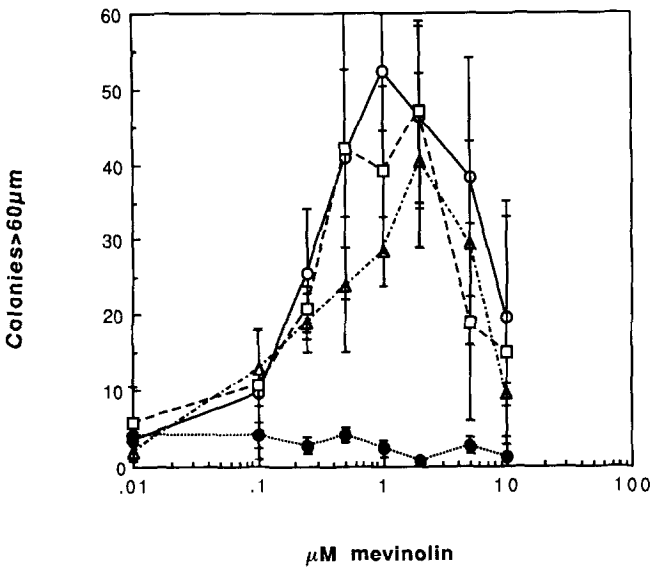

Fig. 1. Stimulatory effect of mevinolin on anchorage independent growth of SW13 cells. Cells were plated in suspension over 1% agarose base layers in the presence of mevinolin alone (0--0), or mevinolin plus 1 mM mevalonic acid (● --●), 15 μg/ml low density lipoproteins (Δ···Δ) or 1 μg/ml dolichol (□ -- □). The number of colonies greater then 60μm was determined by image analysis 5 days after plating. Bars represent the standard deviation of triplicates.

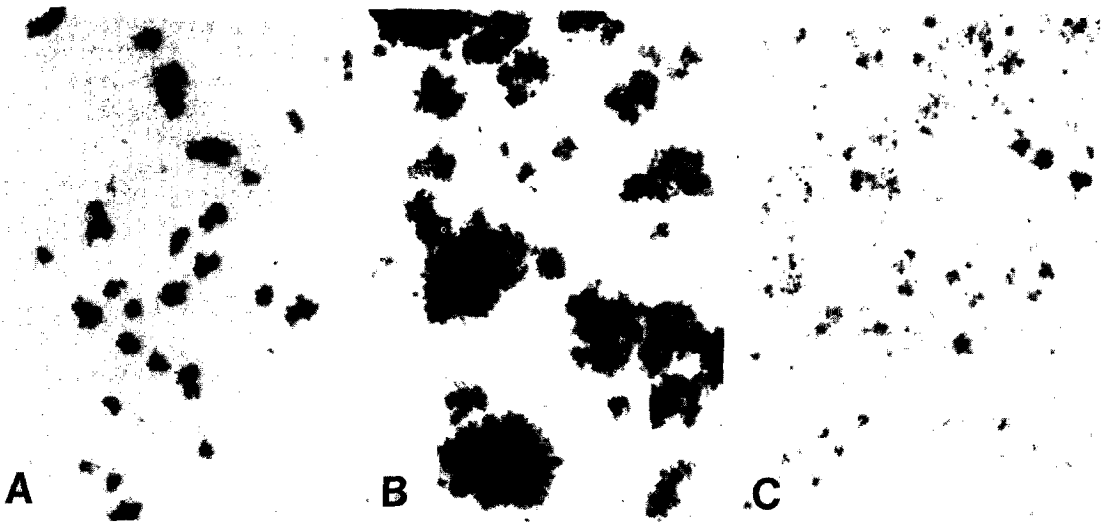

Fig. 2. (A) Untreated culture of SW13 cells. (B) Culture grown in the presence of 2.5 μM mevinolin. (C) SW13 cells treated with 2.5 μM mevinolin plus 1 mM mevalonic acid. Magnification X 182.

1 μg/ml dolichol (Fig. 1). In contrast, supplementation of the cells with 1 mM mevalonate completely abolished the proliferative response to mevinolin (Figs. 1,2). Addition of mevalonate did not inhibit the response of the cells to TGFe (Fig. 3).

To examine the effect of concentrations of mevinolin that induce cell growth on HMG CoA reductase, the activity of the enzyme was measured in suspension cultures undergoing a proliferative response to

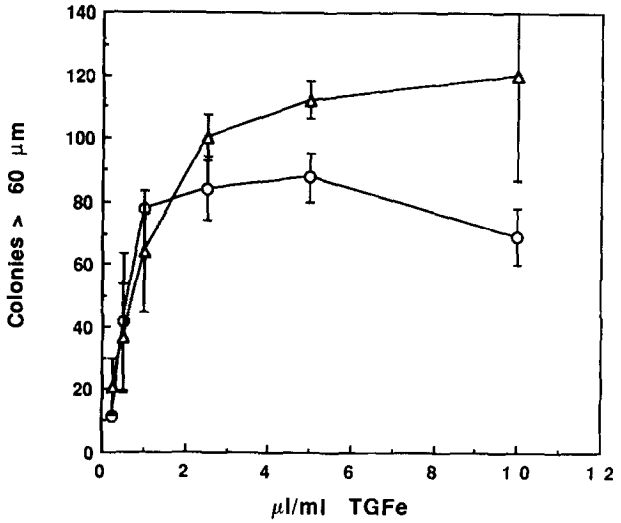

Fig. 3. Lack of inhibition by mevalonic acid of the response of SW13 cells to TGFe. Bovine kidney-derived TGFe was added to cultures of SW13 cells with (Δ--Δ) or without (O--O) 1 mM mevalonic acid. Bars represent the range of duplicates.

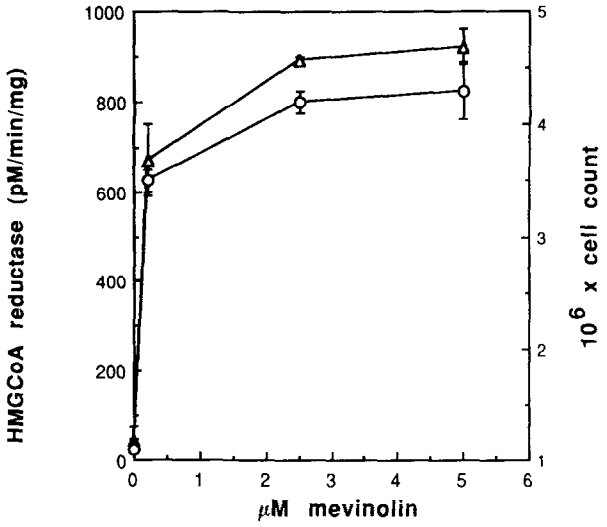

Fig. 4. Assay of HMG CoA reductase activity in SW13 cells treated with mevinolin. Cells were harvested from suspension cultures and the count (o--o) and enzyme activity (Δ--Δ) were determined. Enzyme activity measurements were corrected for counting efficiency and recovery from the ion exchange columns. Background counts were determined by adding 100 μM mevinolin to the reaction mixture and were subtracted from the experimental values. Bars represent the range of duplicates.

the drug. In these cells, activity of the enzyme was markedly increased in comparison to untreated cells (Fig. 4). The dose-response profile of enzyme induction roughly paralleled that of cell number (Fig. 4).

DISCUSSION

The results show that low micromolar concentrations of mevinolin stimulate anchorage independent growth of SW13 cells. Higher concentrations inhibit cell growth. The stimulatory effect of mevinolin on colony formation appeared to be related to the ability of the drug to inhibit HMG CoA reductase. Thus, supplementation of mevinolin-treated cultures with 1 mM mevalonate completely abolished the proliferative response. The response of SW13 cells to TGFe was not inhibited by mevalonate supplementation, indicating that lack of colony formation was not due to generalized toxicity of this compound.

A paradoxical increase in HMG CoA reductase activity was observed in mevinolin treated cells. Experiments with the related compound compactin (9) have shown that cells undergo a compensatory response to such drugs by synthesis of new enzyme. The newly synthesized enzyme is, nevertheless, inhibited by the drug (9). This induction phenomenon may

in part explain the apparent difference between the concentrations of mevinolin needed to produce an effect in SW13 cells and the potency of the drug as an inhibitor of HMG CoA reductase in cell-free assays (Ki 0.6 nM), since more drug would be needed to overcome the compensatory increase in enzyme synthesis.

Previous studies describing antiproliferative effects of sterol biosynthesis inhibitors have suggested that a non-sterol metabolite of mevalonic acid is required for DNA replication (2). In our experiments with mevinolin, depletion of cellular mevalonate stimulates proliferation. The reason for this apparent contradiction is probably related to the experimental conditions of anchorage dependence versus independence, the presence of 10% serum in our studies and possibly the epithelial origin of SW13 cells compared with previous studies in which fibroblasts and smooth muscle cells were examined. In our system, the maximum response to mevinolin is only 50% of that seen with the polypeptide growth factor TGFe. This finding suggests that mevinolin may stimulate proliferation by depletion of one or more cellular growth inhibitors derived from mevalonic acid. In this regard, it is interesting that a number of cellular proteins are modified by isoprenoid groups derived from mevalonic acid (10). A recent report (11) suggested that p21 Ras requires the addition of a farnesyl group derived from mevalonate for membrane association and biological activity. It is possible that the action of proteins involved in cellular growth regulation may be accessible to pharmacological intervention by modification of post-translational processing such as the attachment of isoprenoid groups.

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