

Regulation of Cholesterol Synthesis in Four Colonic Adenocarcinoma Cell Lines

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ABSTRACT: Colon tumor cells, unlike normal human fibroblasts, exhibited an uncoupling of low density lipoprotein (LDL)-derived cholesterol from cellular growth, when endogenous cholesterol synthesis was inhibited by mevinolin, a hydroxymethylglutaryl-CoA reductase (HMG-CoAR) competitive inhibitor [Fabricant, M., and Broitman, S.A. (1990) *Cancer Res.* 50, 632-636]. Further evaluation of cholesterol metabolism was conducted in two undifferentiated (SW480, SW1417) and two differentiated (HT29, CACO2) colonic adenocarcinoma (adeno-CA) cell lines and an untransformed human fibroblast, AG1519A. Cells grown in monolayer culture to near subconfluency were used to assess endogenous cholesterol synthesis by ¹⁴C-acetate incorporation, in response to the following treatments in lipoprotein-deficient serum (LPDS)-supplemented minimum essential medium (MEM): LPDS alone, LDL, mevinolin, mevinolin with LDL, and 25-hydroxy-cholesterol (25-OH-CH). Complete fetal bovine serum (FBS)-supplemented MEM was used as control. All colon tumor lines exhibited similarly high endogenous cholesterol synthesis in both FBS and LPDS relative to the fibroblasts which demonstrated low basal levels in FBS and maximal synthesis in LPDS. LDL treatment did not inhibit cholesterol synthesis in colon tumor cells, but suppressed that in the fibroblast by 70%. Sterol repression of cholesterol synthesis mediated by 25-OH-CH occurred in all cells. Mevinolin caused a reduction in cholesterol synthesis in the colonic cancer cell lines, which was not further decreased by concurrent addition of LDL. In contrast, in mevinolin-treated fibroblasts, LDL further inhibited cholesterol synthesis. When the effect of cell density on cholesterol synthesis regulation was evaluated under conditions of sparse density in SW480 and SW1417, results indicated that (i) basal rates of cholesterol synthesis were higher, (ii) LDL inhibited cholesterol synthesis more effectively, and (iii) mevinolin or 25-OH-CH had a more pronounced effect than in subconfluent cells. Evaluation of LDL receptor activity through ¹²⁵I-LDL binding and internalization studies demonstrated LDL receptor expression was reduced by 37% in normal density cells relative to the low density cultures. In contrast to cholesterol synthesis, exogenous LDL could in-

hibit LDL receptor activity at both densities. Thus subconfluent growing colonic adenoCA cell lines retain the capacity for sterol repression, but, in contrast to normal fibroblasts, exhibit a high endogenous cholesterol synthesis which LDL cannot regulate. *Lipids* 30, 1083-1092 (1995).

Cholesterol is required for growth of cells (1). This sterol may be obtained either by *de novo* synthesis from acetate, which is catalyzed by the enzyme 3-hydroxymethylglutaryl-CoA reductase (HMG-CoAR), or *via* uptake of exogenous cholesterol from the environment, primarily as low density lipoprotein (LDL) (2). A relationship between the neoplastic state and loss of feedback inhibition in cholesterol metabolism was first suggested by Siperstein *et al.* (3), who described a failure of dietary cholesterol to regulate *de novo* synthesis in hepatoma and prehepatoma cells. Rapidly proliferating cancer cells may have a higher cholesterol requirement for membrane or synthetic purposes; consequently, these increased demands for cholesterol can be met either by increasing endogenous synthesis (4,5) or uptake (6-13) of exogenous cholesterol as LDL, or both (14).

The rate-limiting step in the cholesterol biosynthetic pathway is catalyzed by HMG-CoAR (15). Increased activity of this enzyme increases its direct product, mevalonate, precursor to sterols and nonsterol isoprenoids, such as ubiquinone, dolichol, and isopentenyl tRNA, which are all necessary for cell growth (16). Mevalonate also incorporates into isoprenylated proteins (17), including such signal transduction participants as ras and other GTP binding proteins (18). An increase in the expression of HMG-CoAR has been described in certain tumors relative to normal tissue (19-21), indicating its importance in cellular growth control.

Cells grown in culture exhibit a preference for cholesterol *via* receptor-mediated uptake of LDL (22). Inhibition of cholesterol synthesis by compactin, a fungal metabolite known to competitively inhibit HMG-CoAR (23), renders growth of fibroblasts, Chinese hamster ovary, and raji lymphoma cells dependent on the LDL receptor (LDLR) (24). When the four lines used in these experiments were grown in lipoprotein-deficient serum (LPDS)-supplemented media in the presence of mevinolin (at a dose which inhibits growth by 50%), LDL was unable to reverse growth inhibition, in direct contrast to the human fibroblast, where growth was totally restored (25).

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Abbreviations: ACAT, acyl cholesterol acyl transferase; adeno-CA, adenocarcinoma; ANOVA, analysis of variance; BSA, bovine serum albumin; FBS, fetal bovine serum; HMG-CoAR, 3-hydroxy-3-methylglutaryl-CoA reductase; 25-OH-CH, 25-hydroxy-cholesterol; LDL, low density lipoprotein (quantities refer to measured or derived protein); LDLR, LDL receptor; LPDS, lipoprotein-deficient serum; MEM, minimum essential medium; PBS, phosphate-buffered saline; TBS, tris-buffered.

It has been demonstrated that small intestinal cells do not use exogenous LDL for growth when endogenous cholesterol synthesis is inhibited by mevinolin, though LDL receptors may be present and this lipoprotein internalized by the cells (26–28). However, discrepancies still exist as to whether or not intestinal cells preferentially use endogenous cholesterol for membrane synthesis (29). In order to further elucidate previous findings, we studied cholesterol metabolism in four colonic adenocarcinoma (adenoCA) cell lines, two of which (HT29, CACO2) differentiate *in vitro* under appropriate conditions to small intestinal cells (30,31). Results suggest a lack of regulation upon endogenous cholesterol synthesis by LDL in subconfluent growing colonic adenoCA cells, similar to the metabolism of a differentiated small intestinal cell phenotype.

MATERIALS AND METHODS

Cell culture. Four human colonic cancer cell lines, SW480, SW1417, HT29, and CACO2, were obtained from the American Type Culture Collection (Rockville, MD). Stock cultures were grown in monolayer in plastic 75 cm² flasks and incubated at 37°C in a 5% CO₂/95% air atmosphere. Culture medium consisted of Eagle's minimal essential medium (MEM) containing antibiotics (penicillin, 200 units/mL; streptomycin, 0.2 mg/mL), glutamine (0.2 mg/mL), and nonessential amino acids and nonessential vitamins both at 10 mL of 100X solution (GIBCO, Grand Island, NY), per 500 mL MEM and supplemented with 10% fetal bovine serum (FBS) (Sigma, St. Louis, MO). Cells were plated at a density of 10⁶ cells per flask, and cultures divided weekly (1:19 or 1:20) using 0.05% trypsin/0.02% disodium EDTA. After removal of media, cells were rinsed with isotonic phosphate-buffered saline (PBS) (pH 7.4), and 4 mL of trypsin added. Flasks were incubated at 37°C until cells began to detach, after which 4–6 mL of fresh medium was added. Cells used in experiments are grown in plastic 35 mm² dishes. Normal human skin fibroblast cells (AG1519A), obtained from the Institute for Medical Research (Camden, NJ), were grown as monolayers in MEM supplemented with antibiotics, glutamine, nonessential amino acids, and 20% FBS. Stock cultures were plated at a density of 1 × 10⁶ per 75 cm² flask and divided weekly (1:4) using trypsin/EDTA. Cell cultures were assayed for the presence of mycoplasma using the Gen-Prove Mycoplasma T.C. 11 Rapid Detection System (San Diego, CA).

Preparation of LPDS. Lipoproteins were removed from FBS by density gradient centrifugation (32). FBS was adjusted to a density of 1.21 g/mL with solid potassium bromide (64.4 g KBr per 200 mL FBS) and centrifuged for 48 h at 5°C at 244,000 × *g*_{max} (45,000 rpm in a 50.2ti rotor in a Beckman L8-70 ultracentrifuge; Beckman Instruments, Fullerton, CA). The top lipoprotein fraction (*d* < 1.21 g/mL) was removed, and the bottom lipoprotein-deficient fraction dialyzed extensively against 0.01% EDTA/double-distilled water (pH 8) and then PBS. The LPDS was sterilized by passage through a 0.22 μ filter.

Preparation of mevinolin. This reagent was kindly provided by A. Alberts of Merck Sharp & Dohme (Rahway, NJ) in the lactone form. Saponification was carried out by adding 0.1 mL absolute ethanol and 0.1 mL of 0.1 N NaOH to 4 mg of mevinolin and by heating at 50°C for two hours. Mevinolin was neutralized with 5% HCl to pH 7.3. Stock solutions (4 mg/mL) were prepared by bringing the volume to 1 mL with dimethyl sulfoxide and stored at –20°C. At the concentrations used, dimethyl sulfoxide was found to have no effect on cell growth. Mevinolin was diluted in MEM to the concentrations used in experiments. All the studies regarding the use of mevinolin were conducted at a 1 μM concentration as published by this laboratory (25).

Protein determinations. Proteins were determined by the bicinchoninic acid reagent method (Pierce Chemical Company, Rockford, IL). A standard curve was prepared using a stock bovine serum albumin standard in sample diluent. Samples and standard are mixed with the protein reagent and absorbance read at 562 nm by spectrophotometry.

Cholesterol synthesis assay. Cells were seeded in six-well (35 mm²) dishes at 20,000 cells/well in triplicate per group studied per experiment, in MEM supplemented media with 10% (SW480, SW1417, and HT29) or 20% (CACO2 and AG1519A) FBS. All cells were grown to a subconfluent to confluent growth, termed normal density (≈80% of the plate was covered after day 5), except where specified as “LD,” meaning low density or sparse growth (≈30% was covered, after day 3). After day 5 (day 3 for LD cultures), cells were then rinsed with Dulbecco's PBS and switched to a LPDS based medium (1 mL/well). After 24 to 48 h, the media was replaced with media containing 5 μCi/mL of 2-¹⁴C-acetate (51 mCi/mole NEN), and the following treatments: (i) FBS (ii) LPDS, the basal media for all subsequent treatments (each of which contain 10% LPDS), (iii) LDL (50 μg protein/mL), (iv) 25-OH-CH (25-OH-CH at 0.5 μg/mL + cholesterol at 16 μg/mL), (v) mevinolin at a 1 μM concentration, and (vi) mevinolin + LDL. After 24-h incubation, the medium was removed and saved; monolayers were harvested in 1 mL 0.1 N NaOH and pooled with their respective saved media to be saponified following the methodology of Brown *et al.* (32). Briefly, these mixtures were treated with 0.5 mL 50% KOH, 3 mL ethanol, and (1,2) ³H-cholesterol (10⁵ cpm, 50 Ci/mole) as internal standard. After saponification at 80°C for two hours, unsaponifiable lipids were extracted three times with petroleum ether followed by two washes with NaOH. Samples were dried with nitrogen gas, resuspended in chloroform, spotted on silica gel “G” thin-layer chromatography plates, and separated using chloroform as a running solvent to isolate the ¹⁴C-cholesterol with appropriate markers. This methodology discriminated 27C sterols from lanosterol and squalene, which had higher R_f values. Although this running system does not resolve cholesterol from other 27C sterols, gas chromatography analysis of the derivatized lipids from the scrapings showed a predominance of cholesterol with some desmosterol and 7α-dehydrocholesterol (data not shown). Cholesterol synthesis was evaluated as nanomoles of

acetate incorporated into cholesterol per mg cellular protein, and these values normalized to the LPDS control within each experiment. Results of challenge with various treatments were expressed as percentage of LPDS control. Data was analyzed by general linear model analysis of variance (ANOVA) to determine significant differences between groups.

¹²⁵I-LDL binding to colonic adenoCA cell lines. The ¹²⁵I-LDL binding assays were conducted using the following methodology adapted from Goldstein *et al.* (33). Cells were seeded at 2.5 and 15 × 10⁴ cells per well in three wells each of six-well plates to generate contrasting conditions of normal, and low density within individual assay plates. After two days' growth in FBS-supplemented MEM, cells were washed with PBS and changed to media supplemented with 10% LPDS, which was prepared in-house from FBS (Sigma) to generate maximal responses in terms of receptor activity. After 24 h, the cells received treatments in LPDS-based MEM for a duration of 24 h. Following treatments, the cells were incubated in 1 mL LPDS/MEM with 5–10 µg/mL ¹²⁵I-LDL (Biomedical Technologies Inc., Stoughton, MA) for five hours. Within an experiment, two wells of a six-well dish are used to measure hot or total binding per treatment at either density state. To control for nonspecific binding, one well received 25- to 40-fold increase of cold LDL (125–200 µg/mL) above the level of the hot LDL in the media. One empty, cell-free, six-well plate was treated with media, washed, treated, and incubated just as the other groups, and used as a blank.

Surface binding. After incubation for five hours, a steady state is reached in which binding, internalization, and degradation of the lipoprotein are equilibrated (33). The cells were placed on an ice bed, and rinsed 5 times with 2 mL ice-cold tris-buffered saline (TBS)-A buffer (50 mM Tris/150 mM NaCl/2 mg/mL albumin at pH 7.4), once with ice-cold TBS buffer (TBS-A with no albumin), and finally incubated with 1.5 mL of dextran sulfate release buffer (50 mM NaCl/10 mM HEPES/4 mg/mL dextran sulfate at pH 7.4) for one hour at 4°C. Polyanionic macromolecules such as dextran sulfate or heparin sulfate cause LDL to be released from its receptor; thus the activity of the released tracer is a measurement of the surface binding capacity for the cells. Following this incubation, 750 µL aliquots were taken for gamma counting.

Internalization. The major count found in this assay represents the LDL that is internalized by the cells, which has not yet been degraded. In fibroblasts this will typically be 80–90% of the total cell associated radioactivity (33). To measure internalization, the cells are returned to the ice bed and washed two times with 2 mL TBS buffer. Then, the monolayers are dissolved in 1.5 mL hot 60°C NaOH (0.1N), and aliquots are taken for gamma counting (750 µL) and protein (20 µL) measurement.

RESULTS

Colon tumor cells were evaluated for their ability to synthesize cholesterol in response to different sterol treatments.

Cells were grown in culture to a subconfluent state to limit any growth-rate dependent effects of replication upon the results. At subconfluency, cells were deprived of exogenous sterols through culture for 24 h in LPDS-based MEM. Under these conditions, fibroblasts depend upon endogenous cholesterol synthesis for growth (2). Cells were then subjected to subsequent treatments. All data is normalized as a percentage of the individual experiments' LPDS control group.

FBS provides cells with a sterol-rich medium, generating a reference point to determine levels of endogenous synthesis under "normal" growth conditions. The colonic cell lines exhibited similarly high basal rates of cholesterol synthesis in both FBS- and LPDS-supplemented media. The fibroblast presented low basal levels of cholesterol synthesis when grown in FBS (Fig. 1A–E), suggesting an increased reliance upon endogenous synthesis in subconfluent colonic adenoCA cells.

LDL is the primary cholesterol source in man (2), and certain cells in culture preferentially derive this sterol from LDL in the serum of culture media, synthesizing cholesterol at a low rate (22). When colonic tumor cells grown in LPDS were treated with this lipoprotein, cholesterol synthesis was not suppressed (Fig. 1A–D), as occurs in the fibroblast (Fig. 1E), indicating an inability of this lipoprotein to regulate endogenous cholesterol synthesis.

Regulation by LDL of HMG-CoAR has been shown to occur both transcriptionally and post-transcriptionally *via* a putative oxysterol intermediate (16). In order to test if colonic tumor cells can demonstrate a functional sterol-mediated suppression of this enzyme, the ability of the oxysterol 25-OH-CH to repress cholesterol synthesis was tested. All cells were found to exhibit a decrease in cholesterol synthesis when treated with this oxysterol in LPDS (Fig. 1A–E), indicating that oxysterol regulatory mechanisms remained functional in these lines.

Most of the work in cholesterol metabolism in human fibroblasts has taken place at a confluent state (35–37) in order to avoid replication-dependent factors upon the results. However, alterations in confluency state can cause certain cultured colonic cell lines such as CACO₂ cells to differentiate and gain small bowel phenotypic characteristics. Therefore, we examined whether cell density had an effect on the ability of sterols to regulate cholesterol synthesis in two lines which are not known to differentiate in culture to a small bowel phenotype, the undifferentiated colonic adenocarcinomas SW480 and SW1417 (Fig. 2). The sterols in FBS and LDL were found to downregulate endogenous cholesterol synthesis in low density growing cells (≈30% of the dish is covered at three days), in direct contrast to subconfluent (≈80% of the dish was covered) cells. Additionally, the rates of *de novo* cholesterol synthesis (see Table 1) in cells cultured at lower densities were higher than at subconfluency, implying a greater demand for endogenously synthesized cholesterol in low density cells. However, while endogenous cholesterol synthesis was inhibited by LDL in normal fibroblasts at both low and normal densities of growth, colon tumor cells were

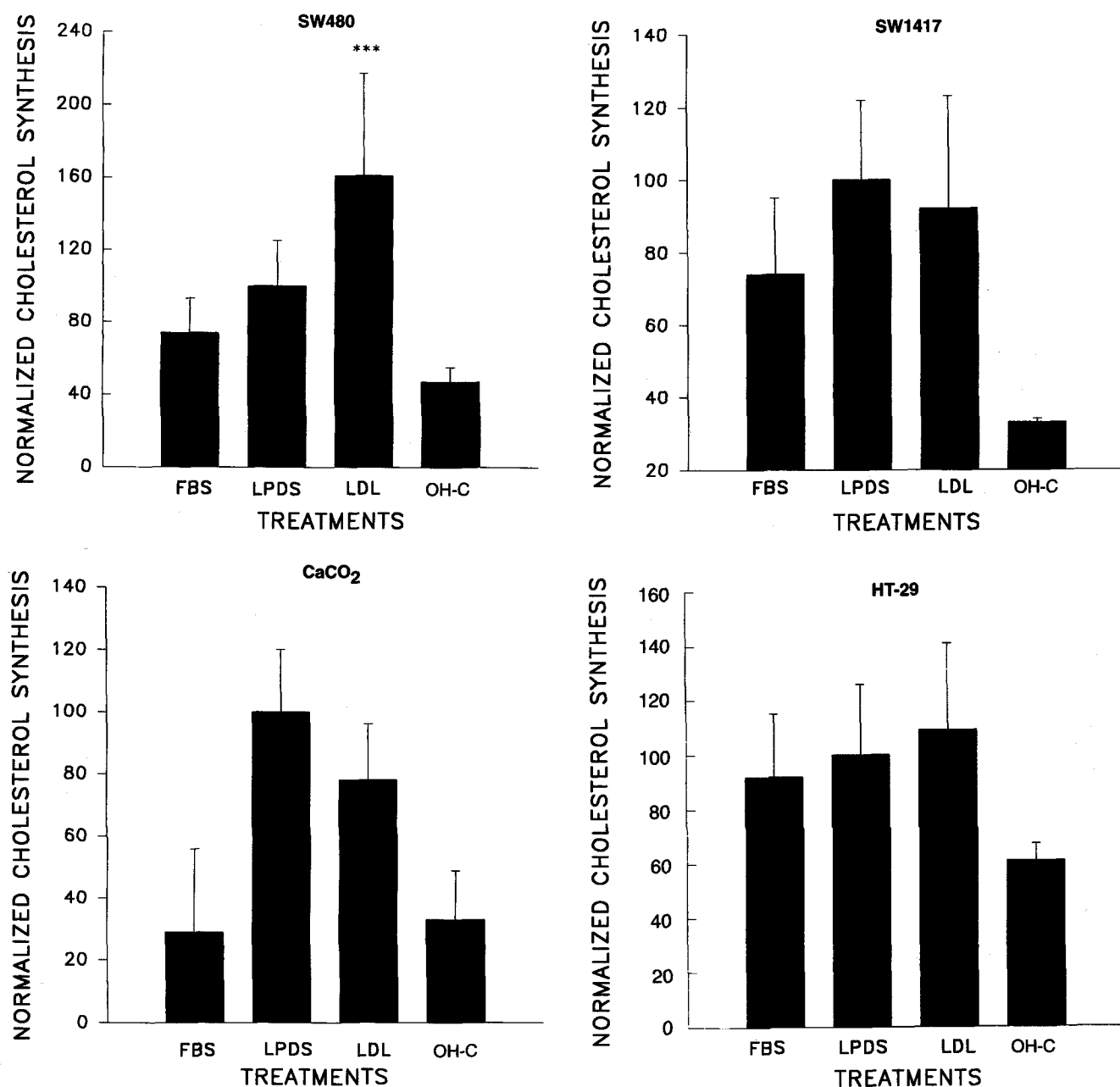


FIG. 1. A-E: (continued on next page).

only repressed by this sterol when growing at low density. It is of interest that control studies have shown the same effects at 1.5 h of treatment, even when the specific activity was diluted to one-third of the original values used in this study (data not shown).

LDLR activity was then analyzed in SW480 cells under these two density culture conditions. LDLR is reported as ng ¹²⁵I-LDL bound per mg cell protein, and the mean of three assays is depicted in Tables 2 and 3. The mean LDLR activity for these cells at both density states in LPDS-supplemented media is low (57 to 90 ng LDL/mg cell protein internalized count) relative to the fibroblast, which has a reported 100 and 600 ng/mg for surface and internalized activity, re-

spectively (33). Thus, as previously speculated (25), this tumor cell line has a lowered LDLR activity relative to fibroblasts. This is consistent with an enhanced cholesterol synthesis rate in these cells, as strictly sterol-regulated genes, such as the receptor, would be repressed by such an increase in intracellular sterols. The LDLR activity between density states across all treatments was not statistically different by ANOVA (α 0.05). Cells were treated with LDL (5, 50, 300 μ g/mL), or 25-OH-CH (3 μ g/mL) in basal LPDS media to evaluate the effect of external sterols on LDLR activity. As seen in Table 2, addition of 5, 50, and 300 μ g/mL LDL resulted in a diminution of LDLR activity at both density states. This difference was statistically significant at both densities between all treated groups and the LPDS controls within each density state (ANOVA P < 0.01; Bonferoni control test for multiple comparisons). Addi-

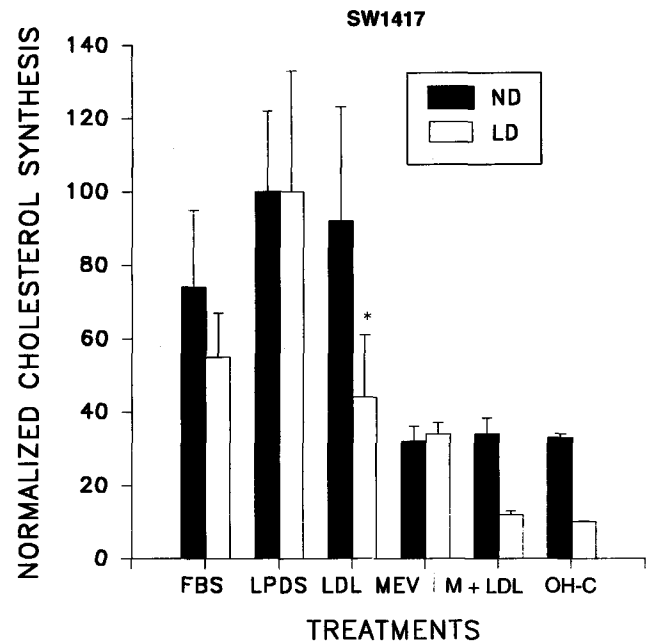
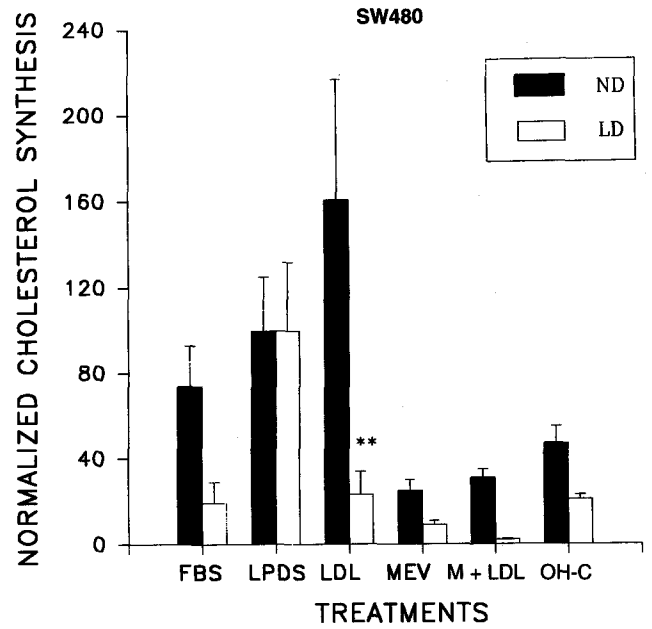
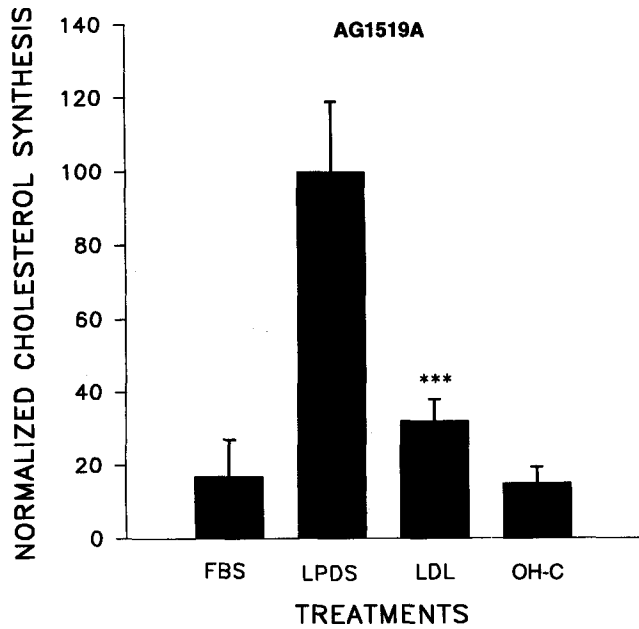


FIG. 1. A-E: Cellular cholesterol synthesis in four colonic adenocarcinoma cell lines (CACO₂, HT29, SW480, SW1417; American Type Culture Collection, Rockville, MD), and a normal human fibroblast AG1519A (Institute for Medical Research Camden, NJ). Cells were grown to subconfluency (80% of the plate was covered), incubated with ¹⁴C-acetate, nonsaponifiable lipids were extracted and subjected to thin-layer chromatography as indicated in the Materials and Methods section. Nanomoles of acetate per mg cellular protein were determined per sample, and these values were normalized to the lipoprotein-deficient serum (LPDS) control within each experiment. Results of challenge with various treatments are expressed as percentage of LPDS control and depicted as bar graphs, where the filled area represents the mean of three different experiments carried out in triplicate. Open areas above the bars represent the standard deviation of the mean. Significance of findings was evaluated by general linear model analysis of variance comparing: low density lipoprotein (LDL) treatments with LPDS controls. FBS, Fetal bovine serum; OH-C, 25-hydroxy-cholesterol.

tion of 25-OH-CH (3 µg/mL) caused significant (*P* < 0.01) inhibition of the LDLR activity vs. the LPDS control group.

In fibroblasts cultured in lipoprotein-deficient medium, inhibition of cholesterol synthesis with the HMG-CoAR inhibitor compactin renders their growth dependent upon LDL-derived cholesterol (24). To assess the effects of a similar HMG-CoAR inhibitor, mevinolin, upon cholesterol metabolism in colonic adenocarcinomas, we determined its impact upon cholesterol synthesis. In order to minimize differences in evaluating the effects of this inhibitor between the lines when analyzing results, the concentration of mevinolin remained the same for all the cells. As shown in Figure 3, mevinolin inhibited cholesterol synthesis in all lines.

This report is consistent with previous findings, where LDL did not reverse growth inhibition imposed by mevinolin (25). In the current study, when the four colonic adenoCA cell lines were grown in the presence of mevinolin, concurrent addition of LDL had no effect upon cholesterol synthesis in the tumor lines (Fig. 3). In contrast, LDL further inhibited cholesterol synthesis in the fibroblast. These effects also were

FIG. 2. A, B: Comparison of cellular cholesterol synthesis in two colonic adenocarcinoma cell lines between subconfluent and lower density culture conditions. SW480 and SW1417 cells were grown to a low density state (30% of the plate was covered), and treated as described for Figure 1. Results are expressed as indicated for Figure 1, except that filled bars represent subconfluent cultures [normal density (ND)] depicted in Figure 1 (C, D), and open bars represent cultures seeded and grown to sparser densities [low density (LD)]; Mev, mevinolin; M + LDL, mevinolin + LDL. See Figure 1 for other abbreviations and company sources.

evaluated in SW480 and SW1417 cells growing at low density (Fig. 2). These cells responded to simultaneous treatment with both mevinolin and LDL by further suppressing cholesterol synthesis, similar to the fibroblast. Additionally, cholesterol synthesis was inhibited to a greater extent by treatment

TABLE 1
Nanomoles of ^{14}C -Acetate Incorporated into Cholesterol per Milligram of Cellular Protein (in 24 h)^a

	Treatment	SW1417	SW480	AG1519A
Low density	LPDS	13.5 ± 5.2	113.1 ± 43.6	17.1 ± 5.7
	LDL	4.2 ± 1.9	37.8 ± 6.4	4.8 ± 0.7
Normal density	LPDS	5.25 ± 2.2	7.35 ± 2.7	7.5 ± 1.9
	LDL	5.9 ± 1.5	12.15 ± 4.5	1.95 ± 0.45

^aCells were grown to a low density state (30% of the plate was covered), or 80% confluent and treated with lipoprotein-deficient serum (LPDS) or low density lipoprotein (LDL) 50 µg/mL (LDL) in the presence of ^{14}C -acetate. Nonsaponifiable lipids were extracted and subjected to thin-layer chromatography as indicated in the Materials and Methods section. Cholesterol synthesis is reported as nanomoles of acetate incorporated per mg cellular protein. Results are expressed as means ± SE of a representative of three experiments, n = 3. SW1417 and SW480 are colonic adenocarcinoma cell lines (American Type Culture Collection, Rockville, MD). AG1519A (Institute for Medical Research, Camden, NJ) is a normal human fibroblast.

TABLE 2
LDLR Activity in SW480 Cells Cultured at Two Different Density Conditions in Response to Various Treatments^a

Treatment	Normal density (internal)	Normal density (surface)	Low density (internal)	Low density (surface)
	Mean SEM	Mean SEM	Mean SEM	Mean SEM
LPDS	57.3 ± 5.9	14.1 ± 5.7	89.8 ± 40.1	20.0 ± 13.9
LDL 5 µg	30.6 ± 8.1	9.0 ± 5.4	31.5 ± 23.6	5.7 ± 4.1
LDL 50 µg	30.6 ± 8.1	4.8 ± 2.6	34.8 ± 20.3	7.3 ± 5.3
LDL 300 µg	10.2 ± 4.1	0.6 ± 0.4	7.6 ± 6.8	0.0 ± 0.0
25-OH-CH 3 µg	18.9 ± 8.3	6.6 ± 3.5	26.1 ± 18.4	10.5 ± 9.2

^aSW480 cells were cultured at normal and low density states, pretreated with LPDS-supplemented media for 24 h, then treated for 24 h with the LPDS-based media alone or supplemented with increasing concentrations of human LDL as indicated or with 25-hydroxy-cholesterol (25-OH-CH) (3 µg/mL). Cells were evaluated as described under methods for their ability to bind (surface activity) and internalize ^{125}I -LDL. Table 1 activity is reported as ng of radiolabeled LDL per mg cellular protein (mean and standard error of six measurements taken over three experiments). See Table 1 for company source and other abbreviations.

with mevinolin and 25-OH-CH as compared to normal density growing subconfluent cells (Fig. 2).

DISCUSSION

Stange and Dietschy (38) first demonstrated an independent regulation between LDL uptake and cholesterol synthesis in rat intestinal epithelium. This work and that of others (26–28,39) led to a proposed model for the compartmentalization of cholesterol metabolism and cellular growth in intestinal cells (40). The present study shows that colonic adenocarcinoma cell lines exhibit different responses in cholesterol synthesis to treatment with: the sterols in FBS, LDL, mevinolin, and mevinolin + LDL. Our results demonstrate that in cultures supplemented with LDL at 50 µg/mL in LPDS-based medium, cholesterol synthesis was not found to be inhibited, as opposed to the fibroblast (Fig. 1). This unresponsiveness to LDL could reflect: (i) relative deficiency in LDLR expres-

TABLE 3
LDLR Activity in SW480 Cells Cultured at Two Different Density Conditions in Response to Various Treatments^a

Treatment	Normal density (internal)	Normal density (surface)	Low density (internal)	Low density (surface)
	Mean SEM	Mean SEM	Mean SEM	Mean SEM
LPDS	100 ± 4.4	100 ± 14.2	100 ± 11.7	100 ± 3.2
LDL 5 µg	50.2 ± 9.2	41.1 ± 18.9	33.9 ± 12.2	19.5 ± 12.3
LDL 50 µg	51.9 ± 10.0	23.3 ± 9.6	26.3 ± 9.0	36.7 ± 5.0
LDL 300 µg	20.1 ± 6.6	6.6 ± 3.6	19.5 ± 13.3	NA ± NA
25-OH-CH 3 µg	29.4 ± 10.9	32.1 ± 12.3	14.2 ± 9.3	52.2 ± 21.3

^aSW480 cells were cultured at normal and low density states, pretreated with LPDS supplemented media for 24 h, then treated for 24 h with the LPDS-based media alone or supplemented with increasing concentrations of human LDL as indicated or with 25-OH-CH (3 µg/mL). Cells were evaluated as described under methods for their ability to bind (surface activity) and internalize ^{125}I -LDL. Activity is reported as percentage of LPDS control for each experiment (mean and standard error of six measurements taken over three experiments). See Tables 1 (also for company source) and 2 for abbreviations.

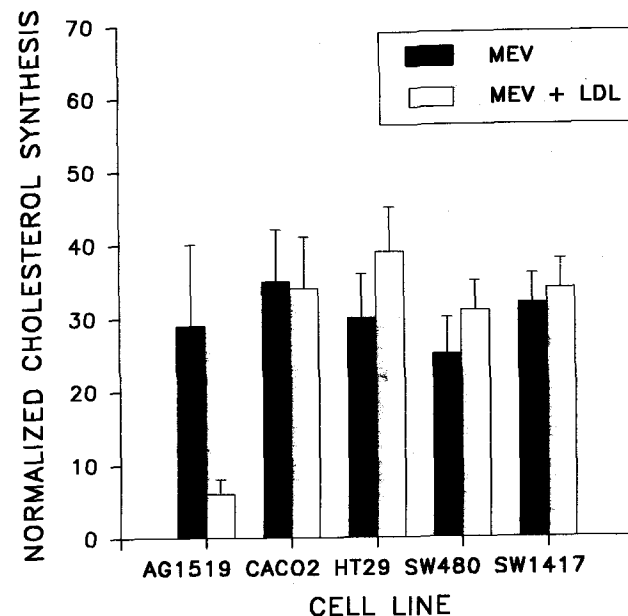


FIG. 3. Cholesterol synthesis in cell lines treated with mevinolin (MEV) or mevinolin and LDL. Cells were cultured as described for Figure 1 and treated with 1 µM mevinolin with (solid bars) or without (open bars) concurrent addition of 50 µg/mL of human LDL. Results are expressed as percentage of LPDS control as described for Figures 1 and 2. See Figure 1 (for company sources also) and 2 for abbreviations.

sion or activity, (ii) an altered compartmentalization, trafficking, or storage of LDL-derived cholesterol into non-regulatory cellular pools, and (iii) a deregulation in the mechanisms by which sterols downregulate HMG-CoAR. The latter hypothesis was deemed unlikely because data indicated normal sterol regulatory function in these cells by 25-OH-CH mediated sterol repression (see Fig. 1A–E).

The inclusion of colonic tumor-derived cell lines into the

development of models designed to help elucidate intestinal cholesterol metabolism necessitates embracing the full spectrum of phenotypic characteristics evidenced by such lines. Cholesterol dynamics for a given tumor cell reflect its current state of (i) differentiation which includes not only "poorly vs. well" differentiated characteristics but also the differences between large vs. small bowel phenotypes, and (ii) cellular density in monolayer culture which may or may not affect such differentiation. The colonic adenoCA cell lines HT-29 and CACO₂ have shown altered regulation of cholesterol metabolism with the state of differentiation (colonic vs. small intestinal) and confluency (28,41,42). When the adenoCA subline HT-29G is undifferentiated, acyl cholesterol acyl transferase (ACAT) activity was found to be absent (43). Consequently, LDL cannot be directed to this enzyme to be stored as cholesterol esters, or used for lipoprotein synthesis as has been hypothesized in small intestinal cells (39); this could have great impact upon the lipoprotein's ability to affect HMG-CoAR. Mevinolin is known to suppress esterification of exogenous, but not endogenous, cholesterol in enterocytes (see Ref. 58). This suggests an effect of mevinolin on the ACAT substrate pool. The inability of LDL to inhibit synthesis may, therefore, be linked to the ability of a cell to turn ACAT on for storage or lipoprotein synthesis. The latter could reflect a differentiation of the colonic adenoCA cell to a small intestinal phenotype.

The correlation of a decrease in serum cholesterol levels with an increased incidence of colorectal cancer has been the focus of many epidemiologic studies (44–47). Normal human (48) and murine (49) colonic epithelium have been shown to be LDLR positive by immunohistochemistry. Expression of the LDLR was found to vary in different portions of the small and large intestine (37,48,49), which may be imposed by the current state of differentiation (40), and growth (41,50,51) of these cells. In fact, immunohistochemical studies from this laboratory have shown that LDLR expression in human normal and neoplastic colon increases with the degree of differentiation of the tissue (48). Other authors found a high proportion of tumors and surrounding normal mucosa to be negative (52) for LDLR (68% of the cases studied); however, no correlation between LDLR expression and serum cholesterol levels, histological grading, or Duke's stage was found.

Consequently, we investigated the state of cellular density and its effect on LDL regulation of endogenous cholesterol synthesis *in vitro* and found a positive correlation. After three days of culture, LDL was capable of inhibiting cholesterol synthesis in cells growing at low density. In contrast, subconfluent cells cultured for five days did not show this regulation (Fig. 2). We cannot dismiss the fact that the incorporation of ¹⁴C-acetate into cholesterol is not a direct measurement of cholesterol synthesis, since differences in intracellular acetate uptake, acetate pools, or activation of acetate to acetyl CoA can occur. Thus, rates of sterol synthesis based on this assay could be regarded as estimates only. Therefore, we conducted a parallel experiment in SW480 cells cultured at the two density states of growth, utilizing ¹⁴C-acetate vs. ¹⁴C-octanoate

as a source of acetyl CoA. Cells preferentially utilize this medium-chain fatty acid as a source of acetyl CoA, *via* its rapid intramitochondrial oxidation, avoiding some of the problems discussed above (53). Results showed that the cells exhibited similar responses to the different treatments when either label was used (data not shown). Nevertheless, since intestinal cells have been reported to prefer ¹⁴C-acetate as a source of acetyl CoA to synthesize cholesterol (53), we used ¹⁴C-acetate and not ¹⁴C-octanoate for our experiments. While it is known that contact inhibited vascular endothelial cells bind LDL with high affinity, without affecting cholesterol metabolism (54), LDLR decrease in number as fibroblasts reach confluency (55). Our results demonstrate that in contrast to the fibroblast, colonic cells exhibited no downregulation of cholesterol synthesis by LDL at subconfluency (Table 1). This variation of cellular density with lipoprotein metabolism also has been shown by many others in intestinal cells in culture (28,40,41) and in neoplastic cells (56,57).

To determine if the culture density-dependent ability of LDL to inhibit cholesterol synthesis was due to a variation in LDLR activity which was concurrently cell density-dependent, the ability of these cultured cell lines to bind and internalize LDL at normal and low densities was assessed. The LDLR activity of LPDS control cultures was 57.3 (normal density) and 89.8 (low density) ng ¹²⁵I-LDL internalized/mg cell protein. These were not statistically different by ANOVA under even nonstringent conditions ($\alpha = 0.05$; $n = 6$). It is unlikely, should subsequent testing determine an actual difference between these groups, that a 36% decrease in activity from low to normal density should confer an LDL unresponsive phenotype in terms of cholesterol synthesis inhibition. Evidence exists for phenotypic change in terms of cholesterol metabolism with changing growth and differentiation states in colonic adenoCA cells. The observed inability of LDL pretreatment to reduce cholesterol synthesis in four colonic adenoCA cell lines cultured at normal density is similar to observations reported by Villiard *et al.* (58) for HT29 cells in stationary vs. exponential phase. This group also found LDLR activity to be more responsive vs. control under exponential (approximately 85%) than stationary (10%) conditions with a dramatic difference in the differentiated "G-" phenotype (58). This contrasts with our findings, which are supportive of a partially independent regulation of the two sterol controlled genes as suggested previously by others (28,38,40). Under conditions where synthesis of C27 sterols was unaffected by LDL in normal density SW480 cells, LDL pretreatment (5 μ g/mL) caused LDLR expression to be reduced to 50 and 34% of the control LPDS treatments in normal and low density cells, respectively. Pretreatment with increased amounts of LDL or 25-OH-CH confirmed that exogenous sterols can be internalized and can cause inhibition of LDLR activity. Although specific degradation of LDL particles was not assessed, the ability of LDL to downregulate its own receptor's activity at each density state is strong evidence that such degradation is taking place even at normal densities where cells are not responsive to cholesterol synthesis inhibition by LDL.

In the fibroblast, the activity of HMG-CoAR and of overall sterol synthesis is regulated according to the rate of cell growth and proliferation (2). However, regulation of the LDLR and HMG-CoAR may not always directly relate to replication, as cell density (59), and standard culture conditions (51) may affect the results. The rates of *de novo* cholesterol synthesis (see Table 1) in cells growing at lower densities were higher than in the subconfluent cells, possibly due to a greater degree of proliferation in the lower density cells. Our laboratory is currently investigating the correlation of LDLR with growth and proliferation in these tumor cells (34).

Treatment of various colonic tumor cell lines with the competitive inhibitor of HMG-CoAR, mevinolin, reduced cholesterol production from acetate in all cells; however, cells cultured at low density were more inhibited by mevinolin treatment than those grown to subconfluence (Fig. 2). Similarly, enhancement of inhibitory action was noted in cells tested at low density relative to subconfluency with other inhibitors (Fig. 2) as 25-OH-CH (nonreceptor-dependent repressor of transcription and enhancer of protein degradation), or LDL (receptor-dependent repressor of transcription and enhancer of protein degradation). These findings are consistent with a possible flux of acetate (and thus mevalonate) toward nonsterol isoprenoids which may be in higher demand in these cells. Inhibitors would thus impact more strongly on cholesterol production. In fact, LDL and mevinolin combined as a treatment in the lower density cells resulted in enhanced inhibition of cholesterol synthesis (Fig. 2) compared to subconfluent densities.

Despite these responses in terms of cholesterol synthetic ability, under these treatments growth is not restored by LDL in intestinal cells inhibited by mevinolin (25,26,28). It is suggested that concurrent treatment with LDL and mevinolin can result in inhibition of HMG-CoAR in such a way that cellular levels of nonsterol mevalonate-derived products may be affected, thereby causing alterations in growth in tumor cells as compared to fibroblasts. This is of considerable interest as the expression of HMG-CoAR is high in tumors of colonic origin (19), and this may affect the expression of active membrane-bound ras oncogene, also found to be high in colon tumors (60–62). Recent work accomplished using a human lung adenoCA cell line has produced similar findings (63); LDL did not reverse a growth inhibition imposed by mevinolin when cells were grown in LPDS. These authors found a high proportion (40%) of HMG-CoAR activity devoted to the generation of nonsterol products in the lung cancer cells, as compared to the 15–20% seen in the fibroblast. Inhibitors of mevalonate synthesis, such as mevinolin, also have proven to inhibit tumor growth in various animal models (64,65). Thus, this inability to utilize exogenous sterol sources for growth in human cells of intestinal origin under conditions of mevalonate inhibition may be of therapeutic significance.

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REFERENCES

1. Chen, H.W. (1984) Role of Cholesterol Metabolism in Cell Growth, *Fed. Proc.* 43(1), 126–130.
2. Brown, M.S., and Goldstein, J.L. (1986) A Receptor-Mediated Pathway for Cholesterol Homeostasis, *Science* 232(4756), 34–47.
3. Siperstein, M.D., Gyde, A.M., and Morris, H.P. (1971) Loss of Feedback Control of Hydroxymethylglutaryl Coenzyme A Reductase in Hepatomas, *Proc. Natl. Acad. Sci. USA* 68(2), 315–317.
4. Betteridge, D.J., Krone, W., Ford, J.M., and Morris, H.P. (1979) Regulation of Sterol Synthesis in Leukaemic Blast Cells: A Defect Resembling Familial Hypercholesterolaemia, *Eur. J. Clin. Invest.* 9(6), 439–441.
5. Clayman, R.V., Bilhartz, L.E., Buja, L.M., Spady, D.K., and Dietschy, J.M. (1986) Renal Cell Carcinoma in the Wistar-Lewis rat: A Model for Studying the Mechanisms of Cholesterol Acquisition by a Tumor *in vivo*, *Cancer Res.* 46(6), 2958–2963.
6. Vitols, S., Peterson, C., Larsson, O., Holm, P., and Aberg, B. (1992) Elevated Uptake of Low Density Lipoproteins by Human Lung Cancer Tissue *in vivo*, *Cancer Res.* 52(22), 6244–6247.
7. Norata, G., Canti, G., Ricci, L., Nicolini, A., Trezzi, E., and Catapano, A.L. (1984) *In vivo* Assimilation of Low Density Lipoproteins by a Fibrosarcoma Tumour Line in Mice, *Cancer Lett.* 25(2), 203–208.
8. Hynds, S.A., Welsh, J., Stewart, J.M., Jack, A., Soukop, M., McArdle, C.S., Calman, K.C., Packard, C.J., and Shepherd, J. (1984) Low-Density Lipoprotein Metabolism in Mice with Soft Tissue Tumours, *Biochim. Biophys. Acta* 795(3), 589–595.
9. Lombardi, P., Norata, G., Maggi, F.M., Canti, G., Franco, P., Nicolini, A., and Catapano, A.L. (1989) Assimilation of LDL by Experimental Tumours in Mice, *Biochim. Biophys. Acta* 1003(3), 301–306.
10. Ho, Y.K., Smith, R.G., Brown, M.S., and Goldstein, J.L. (1978) Low-Density Lipoprotein (LDL) Receptor Activity in Human Acute Myelogenous Leukemia Cells, *Blood* 52(6), 1099–1114.
11. Gal, D., MacDonald, P.C., Porter, J.C., and Simpson, E.R. (1981) Cholesterol Metabolism in Cancer Cells in Monolayer Culture. III. Low-Density Lipoprotein Metabolism, *Int. J. Cancer* 28(3), 315–319.
12. Vitols, S., Gahrton, G., Ost, A., and Peterson, C.O. (1984) Elevated Low Density Lipoprotein Receptor Activity in Leukemic Cells with Monocytic Differentiation, *Blood* 63(5), 1186–1193.
13. Vitols, S., Gahrton, G., Bjorkholm, M., and Peterson, C. (1985) Hypocholesterolaemia in Malignancy Due to Elevated Low-Density-Lipoprotein-Receptor Activity in Tumour Cells: Evidence from Studies in Patients with Leukaemia, *Lancet* 2(8465):1150–1154.
14. Rudling, M.J., Angelin, B., Peterson, C.O., and Collins, V.P. (1990) Low Density Lipoprotein Receptor Activity in Human Intracranial Tumors and Its Relation to the Cholesterol Requirement, *Cancer Res.* 50(3):483–487.
15. Rodwell, V.W., Nordstrom, J.L., and Mitschelen, J.J. (1976) Regulation of HMG-CoA Reductase, *Adv. Lipid Res.* 14:1–74.
16. Goldstein, J.L., and Brown, M.S. (1990) Regulation of the Mevalonate Pathway, *Nature* 343, 425–430.
17. Schmidt, R.A., Schneider, C.J., and Glomset, J.A. (1984) Evidence for Post-Translational Incorporation of a Product of

- Mevalonic Acid into Swiss 3T3 Cell Proteins, *J. Biol. Chem.* 259(16), 10175–10180.
18. Maltese, W.A. (1990) Post-Translational Modification of Proteins by Isoprenoids in Mammalian Cells, *FASEB J.* 4(15), 3319–3328.
 19. Maltese, W.A. (1983) 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase in Human Brain Tumors, *Neurology* 33(10), 1294–1299.
 20. Engstrom, W., and Schofield, P.M. (1987) Expression of the 3-Hydroxy-3-Methylglutaryl Coenzyme A-Reductase and LDL-Receptor and LDL-Receptor Genes in Human Embryonic Tumours and in Normal Foetal Tissues, *Anticancer Research* 7(3 Pt B), 337–342.
 21. Bennis, F., Favre, G., Le Gaillard, F., and Soula, G. (1993) Importance of Mevalonate-Derived Products in the Control of HMG-CoA Reductase Activity and Growth of Human Lung Adenocarcinoma Cell Line A549, *Int. J. Cancer* 55(4), 640–645.
 22. Goldstein, J.L., and Brown, M.S. (1977) The Low-Density Lipoprotein Pathway and Its Relation to Atherosclerosis, *Ann. Rev. Biochem.* 46, 897–930.
 23. Endo, A. (1992) The Discovery and Development of HMG-CoA Reductase Inhibitors, *J. Lipid Res.* 33(11), 1569–1582.
 24. Goldstein, J.L., Helgeson, J.A.S., and Brown, M.S. (1979) Inhibition of Cholesterol Synthesis with Compactin Renders Growth of Cultured Cells Dependent on the Low Density Lipoprotein Receptor, *J. Biol. Chem.* 254(12), 5403–5409.
 25. Fabricant, M., and Broitman, S.A. (1990) Evidence for Deficiency of Low Density Lipoprotein Receptor on Human Colonic Carcinoma Cell Lines, *Cancer Res.* 50(3), 632–636.
 26. Reimann, F.M., Herold, G., Schneider, A., and Stange, E.F. (1991) Compartmentalization of Cholesterol Metabolism and Cellular Growth in Cultured Intestinal Crypt Cells, *Biochim. Biophys. Acta* 1085(3), 315–321.
 27. Fellermann, K., Reimann, F.M., Herold, G., and Stange, E.F. (1992) Mevinolin, A Competitive Inhibitor of Hydroxymethylglutaryl Coenzyme A Reductase, Suppresses Enterocyte Esterification of Exogenous But Not Endogenous Cholesterol, *Biochim. Biophys. Acta* 1165(1), 78–83.
 28. Reimann, F.M., Herold, G., Grosshans, I., Rogler, G., Fellermann, K., and Stange, E.F. (1992) Regulation of Cholesterol Metabolism and Low-Density Lipoprotein Binding in Human Intestinal Caco-2 Cells, *Digestion* 51(1), 10–17.
 29. Viallard, V., Castan, I., Trocheris, V., and Lacombe, C. (1992) Fate of Exogenous and Newly Synthesized Cholesterol in Intestinal Cell Lines, *Int. J. Biochem.* 24(8), 1315–1321.
 30. Zweibaum, A., Pinto, M., Chevalier, G., Bussaulx, E., Triadoo, N., Lacroix, B., Haffen, K., Brun, J.L., and Rousset, M. (1985) Enterocytic Differentiation of a Subpopulation of the Human Colon Tumor Cell Line HT-29 Selected for Growth in Sugar-Free Medium and Its Inhibition by Glucose, *J. Cell Physiol.* 122(1), 21–29.
 31. Chantret, I., Barbat, A., Dussaulx, E., Brattain, M.G., and Zweibaum, A. (1988) Epithelial Polarity, Villin Expression, and Enterocytic Differentiation of Cultured Human Colon Carcinoma Cells: A Survey of Twenty Cell Lines, *Cancer Res.* 48(7), 1936–1942.
 32. Brown, M.S., Faust, J.R., and Goldstein, J.L. (1978) Induction of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity in Human Fibroblasts Incubated with Compactin (ML-236B), A Competitive Inhibitor of the Reductase, *J. Biol. Chem.* 253(4), 1121–1128.
 33. Goldstein, J.L., Basu, S.K., and Brown, M.S. (1983) Receptor-Mediated Endocytosis of Low-Density Lipoprotein in Cultured Cells, *Methods in Enzymol.* 98, 241–260.
 34. Wilkinson, J., and Broitman, S. (1993) Effect of Low Density Lipoprotein (LDL) upon Proliferation in Three Human Adenocarcinoma Cell Lines, in *Proceedings of the American Association for Cancer Research*, Vol. 34, p. 55.
 35. Brown, M.S., Dana, S.E., and Goldstein, J.L. (1975) Cholesterol Ester Formation in Cultured Human Fibroblasts. Stimulation by Oxygenated Sterols, *J. Biol. Chem.* 250(10):4025–4027.
 36. Goldstein, J.L., and Brown, M.S. (1974) Binding and Degradation of Low Density Lipoproteins by Cultured Human Fibroblasts. Comparison of Cells from A Normal Subject and from a Patient with Homozygous Familial Hypercholesterolemia, *J. Biol. Chem.* 249(16), 5153–5162.
 37. Brown, M.S., Dana, S.E., and Goldstein, J.L. (1973) Regulation of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity in Human Fibroblasts by Lipoproteins, *Proc. Natl. Acad. Sci. (USA)* 70(7), 2162–2166.
 38. Stange, E., and Dietschy, J.M. (1983) Cholesterol Synthesis and Low Density Lipoprotein Uptake Are Regulated Independently in Rat Small Intestinal Epithelium, *Proc. Natl. Acad. Sci. (USA)* 80(18), 5739–5743.
 39. Safanova, I.G., Sviridov, D.D., Roytman, A., Rytikov, F.M., Dolgov, V.V., Nano, J.L., Rampal, P., and Repin, V.S. (1993) Cholesterol Uptake in the Human Intestine. Hypo- and Hyperresponsiveness, *Biochim. Biophys. Acta* 1166(2–3), 313–316.
 40. Suckling, K.E., and Stange, E.F. (1985) Role of Acyl-CoA: Cholesterol Acyltransferase in Cellular Cholesterol Metabolism, *J. Lipid Res.* 26, 647–671.
 41. Viallard, V., Lacombe, C., Trocheris, V., Tabacik, C., and Aliaou, S. (1990) Metabolism of Low-Density Lipoprotein in Differentiated and Undifferentiated HT29 Colon Cancer Cells, *Int. J. Cancer* 46(2), 320–325.
 42. Nano, J.L., Barbaras, R., Negrel, R., and Rampal, P. (1986) Regulation of Cholesterol Synthesis and Binding of Lipoproteins in Cultured Rat Intestinal Epithelial Cells, *Biochim. et Biophys. Acta* 876(1), 72–789.
 43. Viallard, V., Lacombe, C., Nebot, C., and Castan, I. (1991) Acyl-CoA: Cholesterol Acyltransferase in HT 29 Cell Subpopulations. Defect of Activity in the Undifferentiated Cells, *Biochim. Biophys. Acta* 1085(2), 265–271.
 44. Broitman, S.A. (1986) Dietary Cholesterol, Serum Cholesterol, and Colon Cancer: A Review, *Advances in Experimental Medicine and Biology* (Poirier, L.A., Newberne, P.M., and Pariza, M.W., eds.) pp. 137–182, Plenum Press, New York.
 45. Winawer, S.J., Flehinger, B.J., Buchalter, J., Herbert, E., and Shike, M. (1990) Declining Serum Cholesterol Levels Prior to Diagnosis of Colon Cancer. A Time-Trend, Case-Control Study, *JAMA* 263(15), 2083–2085.
 46. Cowan, L.D., O'Connell, D.L., Criqui, M.H., Barrett-Connor, E., Bush, T.L., and Wallace, R.B. (1990) Cancer Mortality and Lipid and Lipoprotein Levels. Lipid Research Clinics Program Mortality Follow-up Study, *Am. J. Epidemiol.* 131(3):468–482.
 47. Kreger, B.E., Anderson, K.M., Schatzkin, A., and Splansky, G.L. (1992) Serum Cholesterol Level, Body Mass Index, and the Risk of Colon Cancer. The Framingham Study, *Cancer* 70(5), 1038–1043.
 48. Sotnikov, A.V., Kupchik, H.Z., Gottlieb, L.S., Steele, G., and Broitman, S.A. (1992) *Gastroenterology* 102, A400.
 49. Fong, L.G., Bonney, E., Kosek, J., and Cooper, A.D. (1989) Immunohistochemical Localization of Low Density Lipoprotein Receptors in Adrenal Gland, Liver, and Intestine, *J. Clin. Invest.* 84(3), 847–856.
 50. Mazzone, T., Basheeruddin, K., Ping, L., and Schick, C. (1990) Relation of Growth- and Sterol-Related Regulatory Pathways for Low Density Lipoprotein Receptor Gene Expression, *J. Biol. Chem.* 265(9), 5145–5149.
 51. Tavangar, K., and Kraemer, F.B. (1988) The Regulation of Hydroxymethylglutaryl-CoA Reductase in Cultured Cells, *Biochim. Biophys. Acta* 970(3), 251–261.

52. Caruso, M.G., Notarnicola, M., Cavallini, A., Guerra, V., Misciagna, G., and Di Leo, A. (1993) Demonstration of Low Density Lipoprotein Receptor in Human Colonic Carcinoma and Surrounding Mucosa by Immunoenzymatic Assay, *Ital. J. Gastroenterol.* 25(7), 361–367.
53. Stange, E.F., and Dietschy, J.M. (1983) Absolute Rates of Cholesterol Synthesis in Rat Intestine *in vitro* and *in vivo*: A Comparison of Different Substrates in Slices and Isolated Cells, *J. Lipid Res.* 24(1), 72–82.
54. Vlodaysky, I., Fielding, P.E., Fielding, C.J., and Gospodarowicz, D. (1978) Role of Contact Inhibition in the Regulation of Receptor-Mediated Uptake of Low Density Lipoprotein in Cultured Vascular Endothelial Cells, *Proc. Natl. Acad. Sci. (USA)* 75(1), 356–360.
55. Kruth, H.S., Avigan, J., Gamble, W., and Vaughan, M. (1979) Effect of Cell Density on Binding and Uptake of Low Density Lipoprotein by Human Fibroblasts, *J. Cell Biol.* 83(3), 588–594.
56. Gal, D., MacDonald, P.C., Porter, J.C., Smith, J.W., and Simpson, E.R. (1981) Effect of Cell Density and Confluency on Cholesterol Metabolism in Cancer Cells in Monolayer Culture, *Cancer Res.* 41(2), 473–477.
57. Rotheneder, M., and Kostner, G.M. (1989) Effects of Low- and High-Density Lipoproteins on the Proliferation of Human Breast Cancer Cells *in vitro*: Differences Between Hormone-Dependent and Hormone-Independent Cell Lines, *Int. J. Cancer* 43(5), 875–879.
58. Villiard, V., Lacombe, C., Trocheris, V., Tabacik, C., and Aliau, S. (1990) Metabolism of Low-Density Lipoprotein in Differentiated and Undifferentiated Ht 29 Colon Cancer Cells, *Int. J. Cancer* 46(2), 320–325.
59. Kenagy, R., Bierman, E.L., and Schwartz, A. (1983) Regulation of Low-Density Lipoprotein Metabolism by Cell Density and Proliferative State, *J. Cell Physiol.* 116(3), 404–408.
60. Michelassi, F., Leuthner, S., Lubienski, M., Bostwick, D., Rodgers, J., and Handcock, M. (1987) Ras Oncogene p21 Levels Parallel Malignant Potential of Different Human Colonic Benign Conditions, *Arch. Surg.* 122(12), 1414–1416.
61. Hand, P.H., Thor, A., Wunderlich, D., Muraro, R., Caruso, A., and Schlom, J. (1984) Monoclonal Antibodies of Predefined Specificity Detect Activated *ras* Gene Expression in Human Mammary Carcinomas, *Proc. Natl. Acad. Sci.* 81, 5227–5231.
62. Hand, P.H., Thor, A., Wunderlich, D., Muraro, R., Caruso, A., and Schlom, J. (1984) *Proc. Natl. Acad. Sci. (USA)* 81, 5227–5231.
63. Bennis, F., Favre, G., Le Gaillard, F., and Soula, G. (1993) Importance of Mevalonate-Derived Products in the Control of HMG-CoA Reductase Activity and Growth of Human Lung Adenocarcinoma Cell Line A549, *Int. J. Cancer* 55(4), 640–645.
64. Maltese, W.A., Defendini, R., Green, R.A., Sheridan, K.M., and Donley, D.K. (1985) Suppression of Murine Neuroblastoma Growth *in vivo* by Mevinolin. A Competitive Inhibitor of 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase, *J. Clin. Invest.* 76(5), 1748–1754.
65. Sumi, S.R., Beauchamp, R.D., Townsend, C.M., Uchida, T., Murakami, M., Rajaraman, S., Ishizuka, J., and Thompson, J.C. (1992) Inhibition of Pancreatic Adenocarcinoma Cell Growth by Lovastatin, *Gastroenterology* 103(3), 982–989.

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