

RESEARCH OUTPUTS / RÉSULTATS DE RECHERCHE

Processing and characterization of the low density lipoprotein receptor in the human colonic carcinoma cell subclone HT29-18

Mazière, J C; Mazière, C; Emami, S; Noel, B; Poumay, Y; Ronveaux, M F; Chastre, E; Porte, H; Barbu, V; Biade, S; Santus, René; Gespach, Christian P.

Published in:
Bioscience reports

Publication date:
1992

Document Version
Publisher's PDF, also known as Version of record

[Link to publication](#)

Citation for published version (HARVARD):

Mazière, JC, Mazière, C, Emami, S, Noel, B, Poumay, Y, Ronveaux, MF, Chastre, E, Porte, H, Barbu, V, Biade, S, Santus, R & Gespach, CP 1992, 'Processing and characterization of the low density lipoprotein receptor in the human colonic carcinoma cell subclone HT29-18: a potential pathway for delivering therapeutic drugs and genes', *Bioscience reports*, vol. 12, no. 6, pp. 483-94.

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Processing and Characterization of the Low Density Lipoprotein Receptor in the Human Colonic Carcinoma Cell Subclone HT29-18: A Potential Pathway for Delivering Therapeutic Drugs and Genes

J. C. Mazière,^{1,4} C. Mazière,¹ S. Emami,² B. Noel,³
Y. Poumay,³ M. F. Ronveaux,³ E. Chastre,² H. Porte,²
V. Barbu,¹ S. Biade,¹ R. Santus,⁴ and C. Gespach^{2,5}

Received August 6, 1992; revised August 27, 1992

Low density lipoprotein (LDL) processing has been investigated in the subcloned human colonic carcinoma cell line HT29-18. LDL binding at 4°C was a saturable process in relation to time and LDL concentration. The K_d for LDL binding was 11 µg/ml. ApoE-free HDL₃ or acetylated LDL did not significantly compete with ¹²⁵I-LDL binding, up to 500 µg/ml. ¹²⁵I-LDL binding was decreased by 70% in HT29-18 cells preincubated for 24 hours in culture medium containing 100 µg/ml unlabelled LDL. Ligand blotting studies performed on HT29-18 homogenates using colloidal gold labelled LDL indicated the presence of one autoradiographic band corresponding to an apparent molecular weight of 130 kDa, which is consistent with the previously reported molecular weight of the LDL receptor in human fibroblasts. At 37°C, ¹²⁵I-LDL was actively internalized by HT29-18 cells and lysosomal degradation occurred as demonstrated by the inhibitory effect of chloroquine. LDL uptake and degradation by HT29-18 cells also resulted in a marked decrease in endogenous sterol synthesis. These data demonstrate that the HT29-18 human cancerous intestinal cells are able to specifically bind and internalize LDL, and that LDL processing results in down-regulation of sterol biosynthesis. Thus, intestinal epithelial cells possess specific LDL receptors that can be exploited to accomplish drug delivery and gene transfer via the receptor-mediated endocytosis pathway.

KEY WORDS: LDL Receptor; internalization; gene and drug therapy; colon cancer.

ABBREVIATIONS: HDL, HCL₃, high density lipoprotein; LDL, low density lipoprotein.

¹Laboratoire de Biochimie, INSERM U312, Faculté de Médecine Saint-Antoine, 27 rue Chaligny, 75012 Paris, France.

²INSERM U55, Hôpital Saint-Antoine, 184 rue du Faubourg Saint-Antoine, Paris, France.

³Unité de Cytologie, Facultés Universitaires Notre Dame de la Paix, Namur, Belgium.

⁴Laboratoire de Physico-Chimie de l'Adaptation Biologique, INSERM U312, Muséum d'Histoire Naturelle, 43 rue Cuvier, 75006, Paris, France.

⁵To whom correspondence should be addressed.

INTRODUCTION

Low density lipoprotein (LDL) is the main cholesterol carrier in plasma. It is catabolized in a variety of tissues by a process involving receptor-mediated endocytosis, resulting in down-regulation of endogenous cholesterol biosynthesis (1, 2). The role of intracellular cholesterol metabolism during cell growth and proliferation has been documented in normal and transformed tissues (3–5). Endogenous cholesterol synthesis is a prerequisite in the active renewal and differentiation of crypt cell progenitors in the small intestine and colon (6, 7). Recent advances in carcinogenesis research indicate that cell division and proliferation are certainly crucial events connected with the risk of cancer, such as familial polyposis coli polyps. This last correlation led us to study the interaction of LDL with HT29-18 human colonic cancer cells, since colorectal cancer is one of the most common types of malignant disease in Western population.

In this paper, we investigated LDL processing and sterol synthesis in HT29-18 cells, a subclone obtained from a human colonic adenocarcinoma (8). The HT-29 cells can be primed for differentiation into enterocyte-like and goblet cells following substitution of glucose for galactose in the culture medium (9, 10). Thus, HT29 cells constitute a suitable model to characterize the molecular and biochemical events related to intestinal cell proliferation and differentiation, and to test new therapeutic approaches against colonic cancers.

Our findings demonstrate specific binding and internalization of LDL by HT29-18 cells. This process is down-regulated after preincubation of the human cancerous intestinal cells in the presence of LDL. We also provide evidence for a lysosomal catabolism of the protein moiety of LDL and for inhibition of sterol synthesis by LDL.

MATERIALS AND METHODS

Materials

^{125}I Na (13–17 Ci/mg) was from Amersham (Buckinghamshire, U.K.). [$^2\text{-}^{14}\text{C}$] sodium acetate (55 mCi/mmol) was from CEA (Saclay, France). Dulbecco's modified minimum essential medium (DMEM) with Earle's salts and fetal calf serum were from Gibco (Grand Island, N.Y., U.S.A.). Ultrosor G was purchased from Industries Biologiques Françaises (Villeneuve la Garenne, France). Silicagel plates F 1500 were from Schleicher and Schuell (Dassel, W. Germany).

Cell Culture

The HT29-18 subclone generously provided by Dr. D. Louvard (Unité de Biologie des Membranes, Institut Pasteur, Paris, France) was cultured in DMEM supplemented with 10% fetal calf serum as previously described (8). Experiments were performed on confluent cell monolayers, approximately 3 to 4 days after seeding.

LDL Preparation and Labelling with ^{125}I Na:

LDL was prepared from normal human serum by 3 steps ultracentrifugation at $105,000 \times g$ in a L8-55 Beckman centrifuge, according to Havel *et al.* (11). The LDL was taken as the 1.024–1.050 density fractions. After dialysis against 0.1 M glycine/0.1 M NaOH buffer (pH = 10), the LDL was iodinated using 1 mCi ^{125}I Na/mg LDL, according to Bilheimer *et al.* (12). After labelling, the LDL was extensively dialyzed against 0.1 M Tris-HCl buffer (pH = 7.4), filtered through $0.45 \mu\text{m}$ Millipore filters and stored at 4°C . The specific radioactivity was about 250–350 dpm/ng of LDL protein. Free ^{125}I iodine in the preparation was under 2%. Protein determination was done by the Lowry method (13).

Preparation of LDL Colloidal Gold Conjugates

Colloidal gold was prepared according to Frens (14) in order to obtain 17 nm gold particles. Conjugation of LDL to gold particles was performed as described by Handley *et al.* (15), and modified by Robenek *et al.* (16).

LDL Binding, Uptake and Degradation

HT29-18 cells were cultured for 24 hours in DMEM medium supplemented with 2% of the serum substitute Ultrosor G, instead of fetal calf serum for maximum LDL receptor expression. Cells were then washed 3 times with phosphate buffered saline (PBS, pH = 7.4). Binding, uptake and degradation of ^{125}I -LDL were studied according to Goldstein and Brown (1). The nonspecific component of ^{125}I -LDL binding, uptake or degradation was determined by addition of a 50-fold excess of unlabelled LDL along with the tracer. The specific component was calculated from the difference between total and nonspecific binding, uptake or degradation. Results are expressed as ng bound LDL/mg of cell protein.

Visualization of the LDL Receptor by Ligand Blotting

The LDL receptor molecule was also identified by the ligand blotting method (17). About 20×10^6 cells were solubilized with 1.5% Triton X 100 according to Friedman *et al.* (18). SDS-polyacrylamide gel electrophoresis was performed on a homogeneous Phastgel 7.5 with a Phastsystem (Pharmacia, Uppsala, Sweden), and proteins were transferred on a nitrocellulose sheet using a semi-dry transfer system (NovaBlot, Pharmacia). The nitrocellulose was then incubated for 2 h at room temperature with $140 \mu\text{g/ml}$ colloidal gold-labelled LDL. The LDL/gold particle ratio was 7/1 as assessed by electron microscopy, thus corresponding to $20 \mu\text{g/ml}$ of unlabelled LDL. The LDL-receptor complex was revealed using the IntenSE BL silver enhancement kit (Janssen Life Sciences Products, Beerse, Belgium).

Lipid Synthesis

Cells were cultured for 24 h in DMEM supplemented with 2% Ultrosor G for maximal induction of sterol synthesis, and then incubated for 4 h at 37°C in the presence of 10 $\mu\text{Ci/ml}$ sodium ^{14}C -acetate. After three washes with PBS, HT29-18 cells were harvested with a rubber policeman. Lipid analysis was performed by thin layer chromatography, as previously described (19). Results are expressed as pmol of ^{14}C -acetate incorporated/mg of cell protein.

All experiments were repeated at least 3 times in duplicate. Where appropriate, statistical analysis was performed using the Student's *t* test.

RESULTS

Figure 1a shows the specific binding of ^{125}I -LDL in HT29-18 cells as a function of the lipoprotein concentration. At 4°C, a plateau was observed for about 40 $\mu\text{g/ml}$ lipoproteins. Scatchard analysis (20) indicated a *K_d* value of 11 $\mu\text{g/ml}$, close to that found by Nano *et al.* in the rat intestinal cell line IRD98 (7). The maximal binding capacity was about 85 ng LDL protein/mg cell protein. The nonspecific binding represented 23% of the total ^{125}I -LDL binding. When HT28-18 cells were incubated at 4°C in the presence of 10 $\mu\text{g/ml}$ ^{125}I -LDL, the plateau was reached after 1 h incubation (Fig. 1b). Thus, LDL binding in

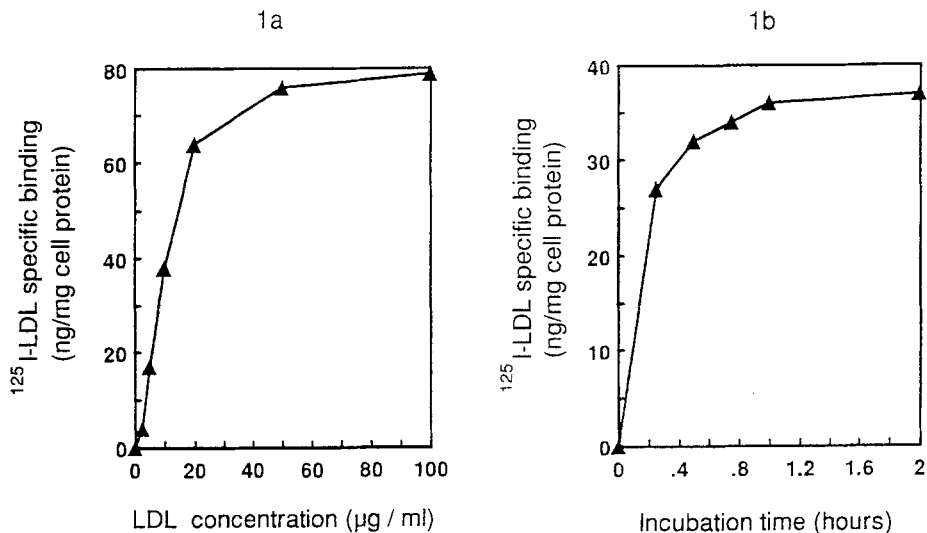


Fig. 1. Specific binding of ^{125}I -LDL to HT29-18 human colonic cancer cells. a: ^{125}I -LDL binding as a function of LDL concentration. Cells were cultured for 24 h in Dulbecco's MEM medium supplemented with 2% Ultrosor G. Then ^{125}I -LDL binding was measured at 4°C in the presence of various concentrations of ^{125}I -LDL ranging from 2.5 to 100 $\mu\text{g/ml}$. Results are expressed as ng of ^{125}I -LDL bound/mg of cell protein (data are means of 4 experiments). b: Specific ^{125}I -LDL binding to HT29-18 cells as function of time. Incubations were performed at 4°C, in the presence of 10 $\mu\text{g/ml}$ ^{125}I -LDL. Results are expressed as ng ^{125}I -LDL bound per mg of cell protein (data are means of 3 experiments).

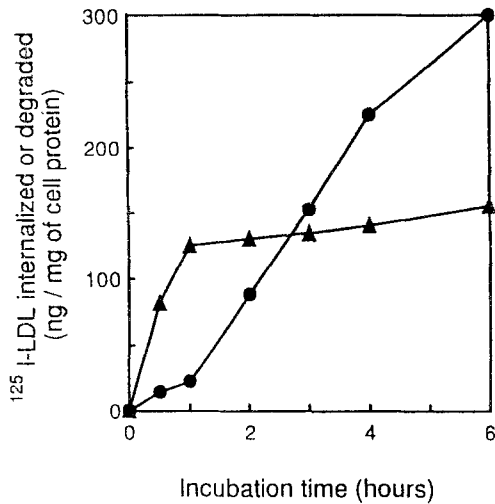


Fig. 2. Kinetics of specific ^{125}I -LDL uptake (\blacktriangle) and degradation (\bullet) in HT29-18 cells. Cells were cultured for 24 h in the presence of Ultroser instead of FCS, as indicated in the legend of Figure 1. Then, the uptake and degradation of LDL were studied at 37°C in the presence of $10\ \mu\text{g}/\text{ml}$ ^{125}I -LDL. Results are expressed as ng ^{125}I -LDL internalized or degraded (data are means of 6 experiments).

HT29-18 cells appeared to be a saturable process, in relation to time and LDL concentration.

Figure 2 shows the kinetics of ^{125}I -LDL internalization and degradation in HT29-18 cells incubated at 37°C . LDL uptake plateaued after 1 h incubation, while LDL degradation was negligible and increased linearly until 4 h incubation. The lysosomal inhibitor chloroquine reduced in a dose-dependent manner ^{125}I -LDL degradation by HT29-18 cells. At the concentration of 1 mM, this drug inhibited degradation by 80% (Figure 3). This result suggests that the protein moiety of LDL is degraded in lysosomes in HT29-18 cells, as previously observed in various cell types, including fibroblasts (21) and hepatocytes (22).

In order to study the specificity of the ^{125}I -LDL binding, competition experiments were performed with increasing concentrations of unlabelled LDL, apo E-free HDL3 or acetylated LDL. Figure 4 shows that ^{125}I -LDL binding was

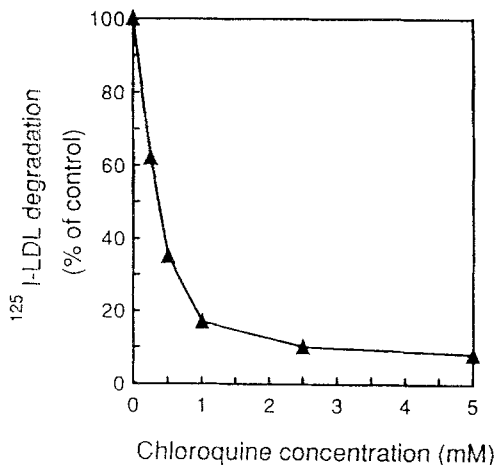


Fig. 3. Effect of chloroquine on ^{125}I -LDL degradation in HT29-18 cells. Cells were incubated for 4 h at 37°C in the presence of $10\ \mu\text{g}/\text{ml}$ ^{125}I -LDL alone or combined with the indicated concentrations of chloroquine. Results are expressed as percent of control (100%: $235 \pm 34\ \text{ng}/\text{mg}$ of cell protein). Data are means of 4 experiments.

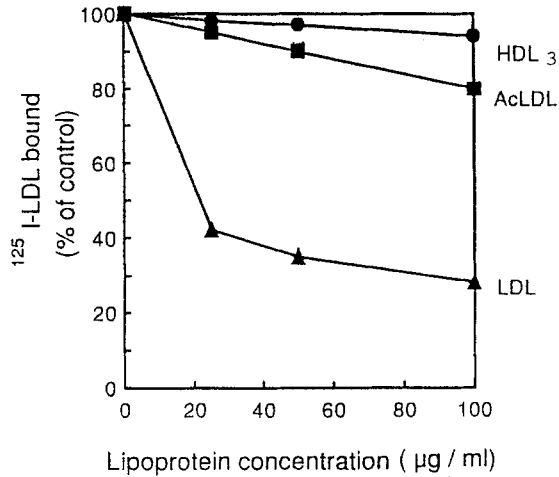


Fig. 4. Competition between unlabelled LDL (▲), unlabelled acetylated LDL (■) or unlabelled HDL₃ (●) with ¹²⁵I-LDL binding in HT29-18 cells. The cells were incubated for 1 h at 4°C in the presence of 10 µg/ml ¹²⁵I-LDL alone or the indicated agents. Results are expressed as percent of control (100%: 42 ± 3 ng/mg of cell protein). Data are means of 4 experiments.

efficiently displaced by unlabelled LDL, resulting in 60% and 80% inhibition of binding at the LDL concentrations of 25 and 250 µg/ml, respectively. In contrast, unlabelled HDL₃ or acetylated LDL did not significantly compete with ¹²⁵I-LDL binding, in the range of the concentrations studied.

Figure 5 displays the ligand-blotting identification of the LDL receptor in HT29-18 cells (lane c). A cell extract of MRC5 normal human fibroblasts has also been studied as a positive control (lane a). It can be observed that in non-reducing conditions, in the absence of β-mercaptoethanol, only one band was obtained, which was located at the same position in the 2 cell types, corresponding to an apparent molecular weight of 130 kDa. This is consistent with the molecular weight of the LDL receptor obtained in human fibroblasts treated under similar conditions (23). It can also be observed in Fig. 5 that when experiments were performed in the presence of an excess of native LDL (at 500 µg/ml), no autoradiographic band was detectable in HT29-18 cells and fibroblasts (lanes d and b). In the presence of 1 mM EDTA, no band was observed either with MRC5 fibroblasts or with HT29 extracts (*data not shown*), indicating that LDL binding by the HT29-18 LDL receptor is dependent upon the presence of free calcium, as previously observed in human fibroblasts (24). When incubated in reducing conditions, in the presence of β-mercaptoethanol, the apparent molecular weight of the LDL receptor in HT29-18 cells shifted to 160 kDa (*data not shown*). Similar findings have been reported for the apo B/E receptor in human fibroblasts (23). Our data indicate that the LDL receptor in HT29-18 human colonic carcinoma cells has the same molecular weight and

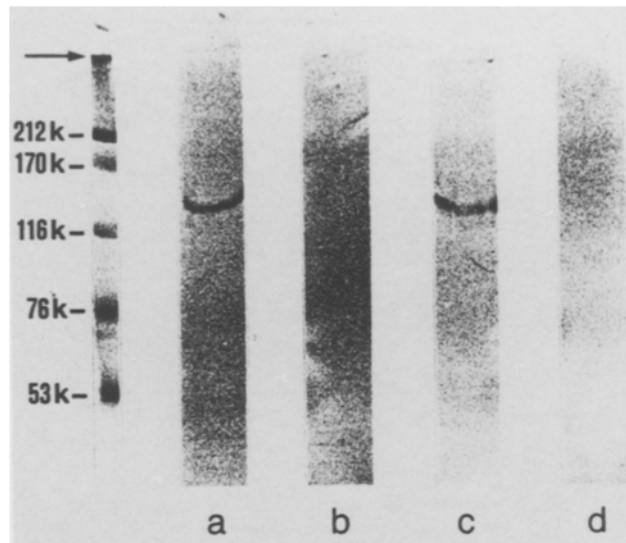


Fig. 5. Ligand blots of the LDL receptor in MRC5 human fibroblasts (a, b) and HT29-18 human colonic carcinoma cells (c, d). Samples obtained from Triton $\times 100$ extracts were subjected to SDS-PAGE in a 7.5% homogeneous Phastgel and transferred electrophoretically to nitrocellulose paper. The blots were incubated with LDL-gold conjugates (140 $\mu\text{g}/\text{ml}$) in the absence (a, c) or in the presence (b, d) of an excess of unlabeled LDL (500 $\mu\text{g}/\text{ml}$). Migrations of molecular weight standards transferred onto the nitrocellulose paper are illustrated on the left.

calcium dependency than the apo B/E receptor in human fibroblasts.

The down-regulation of the LDL receptor by exogenous LDL was also investigated in HT29-18 cells incubated for 24 h in culture medium supplemented with increasing concentrations of unlabelled LDL. The LDL treatment resulted in a dose-dependent decrease of ^{125}I -LDL binding (Figure 6). Half-maximal and maximal down-regulation were respectively observed at the concentrations of unlabelled LDL of 25 and 250 $\mu\text{g}/\text{ml}$. The effect of unlabelled LDL incubated for 24 h on lipid synthesis in HT29-18 cells is presented in Table 1. It can be observed that LDL induced a dose-dependent decrease in sodium ^{14}C -acetate incorporation into sterols (38% and 66% decrease for LDL at 10 and 50 $\mu\text{g}/\text{ml}$, respectively). In contrast, incorporation of the precursor into triacylglycerols or total phospholipids was much less affected.

DISCUSSION

There are only few data concerning the LDL processing by intestinal epithelial cells. In rats, specific LDL binding has been described by Suzuki *et al.* in enzyme-dispersed mucosal cells (25), and more recently by Nano *et al.* in the

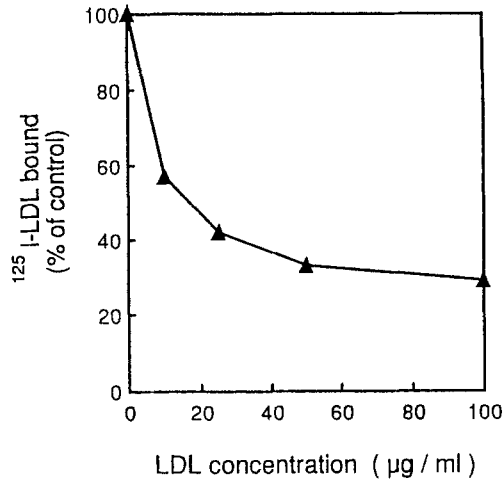


Fig. 6. Down-regulation by LDL of specific ¹²⁵I-LDL binding in human colonic cancer cells HT29-18. After 24 h culture in DMEM supplemented with 2% Ultrosor G and the indicated concentrations of LDL, HT29-18 cells were washed 3 times with PBS and incubated for 1 h at 4°C in the presence of 10 µg/ml ¹²⁵I-LDL. Results are expressed as percent of control (100%: 40 ± 7 ng/mg of cell protein). Data are means of 3 experiments.

epithelial cell line IRD 98 established from explants of fetal small intestine (7). In the present work, we clearly demonstrate that the human cancerous colonic HT29-18 cells can bind, internalize and degrade LDL in a specific manner. The specificity of the LDL receptor in HT29-18 cells is illustrated by the absence of competition between the ¹²⁵I-LDL binding sites and apo E-free HDL₃ or acetylated LDL. Internalization and lysosomal degradation occurs, as dem-

Table 1. Effect of LDL on lipid synthesis from sodium ¹⁴C-acetate in HT29-18 cells

Addition	¹⁴ C-acetate incorporation into:		
	Sterols	Triacylglycerols	Phospholipids
None	1193 ± 145	1801 ± 252	3872 ± 508
LDL,			
10 µg/ml	698 ± 89***	1514 ± 195	3340 ± 455
25 µg/ml	455 ± 67***	1295 ± 144*	2989 ± 308
50 µg/ml	412 ± 55***	1187 ± 156*	2626 ± 325

* Cell monolayers were preincubated for 24 h in the absence or presence of 10 to 50 µg LDL/ml in DMEM supplemented with 2% Ultrosor G. Sodium ¹⁴C-acetate (10 µCi/ml) was added for 4 h at 37°C. Lipid analysis was performed by thin layer chromatography. Results are means of 6 experiments ± s.d. *: $p < 0.05$; ***: $p < 0.001$ by the Student's *t* test.

onstrated by the decrease in ^{125}I -labelled peptides released in the incubation medium and the intracellular accumulation of ^{125}I -LDL in the presence of chloroquine. Moreover, post-receptor events took place, as demonstrated by the down-regulation of ^{125}I -LDL binding and of sterol biosynthesis in HT29-18 cells cultured in the presence of LDL. Finally, the existence of the receptor for LDL in HT29-18 cells is directly demonstrated by ligand blotting studies.

At the present time, the pathophysiological significance of LDL processing in carcinoma-derived colonic epithelial cells, and its occurrence in normal human intestine remained undefined. Although the subclone examined in this study was obtained from a primary colonic carcinoma, there is evidence that cells of the HT29 line resemble normal human intestinal epithelial cells with respect to several morphologic, biochemical, functional and immunological criteria. These include villin and intestinal brush border enzyme synthesis, expression of vasoactive intestinal peptide receptors, EGF and $\alpha 2$ -adrenergic receptors, and preservation of mucin-associated antigens (9, 10, 26, 27). Undifferentiated HT29 cells proliferate as unpolarized layers in a standard culture medium and undergo enterocyte or goblet cell differentiation after glucose privation or butyrate treatment (29–30). This cell line therefore constitute a powerful model in the study of LDL and cholesterol metabolism during the maturation and the functional differentiation of human epithelial cells. The developmental pattern of the LDL pathway in normal intestinal epithelial cells remains to be explored in view of the dietary changes occurring in the suckling newborn and at the weaning (30). It is therefore tempting to suggest that milk lipoproteins and dietary proteins can exert a physiological regulation on the processing of LDL in intestine.

HT29-18 cells also appear to be an interesting model to study the targeting and transfer of genes and some therapeutic agents *via* the LDL receptor pathway. Several epidemiological and laboratory animal model studies indicate that the aetiology of colon cancer is multifactorial and complex (27). Surgical excision has been considered as the only effective treatment for colon cancer. However, the prognosis of patients with colorectal cancer remains poor, mainly due to the occurrence of metastatic invasions and resistance to chemotherapy involving multiresistance gene expression and other mechanisms (31–36). Gene-based therapy *via* the LDL receptor-mediated pathway, using LDL covalently linked to DNA-binding structures such as polyamines, polylysines or other conjugates will allow the delivery of genetic informations encoding anti-metastatic, anti-oncogenic functions, such as tumor suppressor proteins or antisens elements. Some studies are also concerned with the role of LDL in the transport of hydrophobic anticancer drugs, especially anticancer porphyrin derivatives (37–41). LDL has been shown to be the most potent vehicle for delivering anticancer porphyrins to cells. Tumors which can be reached by external or endoscopic irradiation, and among them colorectal cancers, are good targets for photodynamic therapy. Studies are now in progress to investigate the different mechanisms involved in the lytic processes related to the delivery of porphyrins to HT29-18 cells through the LDL pathway.

ACKNOWLEDGEMENTS

J. C. Mazière and C. Gespach thank La Ligue Nationale Contre Le Cancer, Comité de Paris, and MRT (grant n° 90G0234) for financial support.

REFERENCES

1. Goldstein, J. L. and Brown, M. S. (1974) Binding and degradation of LDL by cultured human fibroblasts: comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *J. Biol. Chem.*, **249**:5153–5162.
2. Goldstein, J. L. and Brown, M. S. (1976) The LDL pathway in human fibroblasts: a receptor-mediated mechanism for the regulation of cholesterol metabolism. *Current. Top. Cell. Regul.*, **11**:147–181.
3. Gal, D., McDonald, 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**:315–319.
4. Mazière, J. C., Mazière, C., Mora, L. and Polonovski, J. (1981) Cholesterol metabolism in normal and SV40 transformed hamster fibroblasts. Effect of LDL. *Biochimie*, **63**:221–226.
5. Fairbanks, K. P., Barbu, V. D., Witte, L. D., Weinstein, I. B. and Goodman, D. S. (1986) Effects of mevinolin and mevalonate on cell growth in several transformed cell lines. *J. Cell. Physiol.*, **127**:216–22.
6. Stange, E. F., Preclick, G., Schneider, A., Alvai, M., and Ditschuneit, H. (1981) Regulation of 3-hydroxy-3-methyl-glutaryl Coenzyme A reductase activity by endogenous sterol synthesis in cultured intestinal mucosa. *Biochim. Biophys. Acta*, **663**:613–620.
7. Nano, J. L., Barbaras, R., Négrel, R., and Rampal, P. (1986) Regulation of cholesterol synthesis and binding of lipoproteins in cultured rat intestinal epithelial cells. *Biochim. Biophys. Acta*, **876**:72–79.
8. Louvard, D., Godefroy, O., Huet, C., Sahuquillo-Merino, C., Robine, S. and Coudrier, E. (1985) *Current Communication in Molecular Biology. Protein Transport and Secretion*, (Gething, M. J., ed.) Cold Spring Harbor Laboratory, pp. 168–173.
9. Pinto, M., Appay, M. D., Simon-Assman, P., Chevalier, G., Dracopoli, N., Fogh, J., and Zweibaum, A. (1982) Enterocytic differentiation of cultured human colon cancer cells by replacement of glucose by galactose in the medium. *Biol. Cell.*, **44**:193–196.
10. Dudouet, B., Robine, S., Sahuquillo-Merino, C., Blair, L., Coudrier, E., and Louvard, D. (1987) Changes in villin synthesis and subcellular distribution during intestinal differentiation of HT29-18 clones. *J. Cell. Biol.*, **105**:359–369.
11. Havel, R. J., Eder, H. A., and Bragdon, J. H. (1955) The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.*, **34**:1345–1353.
12. Bilheimer, D. W., Eisenberg, S., and Levy, R. I. (1972) The metabolism of very low density lipoproteins, I. Preliminary in vivo and in vitro observations. *Biochim. Biophys. Acta*, **260**:212–221.
13. Lowry, O. M., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**:265–275.
14. Frens, G. (1973) Controlled nucleation for the regulation of the particle size in monodispersed gold solutions. *Nature Phys. Sci.*, **241**:20–22.
15. Handley, D. A., Arbeeny, C. M., Witte, L. D. and Chien, S. (1981) Colloidal gold-low density lipoprotein conjugates as membrane receptor probes. *Proc. Natl. Acad. Sci. USA*, **78**:368–371.
16. Robenek, H., Rassat, J., Hesz, A., and Grünwald, J. (1982) A correlative study of the topographical distribution of the receptors for low density lipoprotein (LDL) conjugated to colloidal gold in cultured human skin fibroblasts employing thin section, freeze-fracture, deep-etching, and surface replication techniques. *Eur. J. Cell. Biol.*, **27**:242–250.
17. Roach, P. D., Zollinger, M., and Noel, S. P. (1987) Detection of the low density lipoprotein (LDL) receptor on nitrocellulose paper with colloidal gold-LDL conjugates. *J. Lipid Res.*, **28**:1515–1521.

18. Freidman, G., Wernette-Hammond, M. E., Hui, D. Y., Mahley, R. W., and Innerarity, T. L. (1987) Characterization of lipoprotein receptors on rat Fu5AH hepatoma cells. *J. Lipid Res.*, **28**:1482-1494.
19. Mazière, C., Mazière, J. C., Mora, L., and Polonovski, J. (1987) Rapid analysis of cellular lipids without extraction. *J. Biochem. Biophys. Meth.*, **14**:267-272.
20. Scatchard, G. (1949) The attraction of proteins for small molecules and ions. *Ann. Acad. Sci.*, **51**:660-672.
21. Goldstein, J. L., Dana, S. E., Faust, J. R., Beaudet, A. L., and Brown, M. S. (1975) Inhibition of the proteolytic degradation of LDL in human fibroblasts by chloroquine, concanavalin A and Triton WR 1339. *J. Biol. Chem.*, **250**:1703-1713.
22. Pangburn, S. H., Newton, R. S., Chang, C. M., Weinstein, D. and Steinberg, D. (1981) Receptor-mediated catabolism of homologous low density lipoproteins in cultured pig hepatocytes. *J. Biol. Chem.*, **256**:3340-3347.
23. Daniel, T. O., Schneider, W. J., Goldstein, J. L. and Brown, M. S. (1983) Visualisation of lipoprotein receptors by ligand blotting. *J. Biol. Chem.*, **258**:4606-4611.
24. Basu, S. K., Goldstein, J. L. and Brown, M. S. (1978) Characterization of the LDL receptor prepared from human fibroblasts. *J. Biol. Chem.*, **253**:3852-3857.
25. Suzuki, N., Fidge, N., Nestel, P., and Yin, J. (1983). Interaction of serum lipoproteins with the intestine. Evidence for specific high density lipoprotein-binding sites on isolated rat intestinal mucosa cells. *J. Lipid Res.*, **24**:253-264.
26. Chastre, E., Emami, S., Rosselin, G., and Gespach, C. (1985) Vasoactive intestinal peptide receptor activity and specificity during differentiation and retro-differentiation of the human colonic cancerous subclone HT29-18. *FEBS Lett.*, **188**:197-203.
27. Chastre, E., Emami, S., and Gespach, C. (1989) Expression of membrane receptors and (proto)oncogenes during the ontogenic development and neoplastic transformation of the intestinal mucosa. *Life Sci.* **44**:1721-1742.
28. Augeron, C., and Laboisie, C. L. (1984) Emergence of permanently differentiated cell clones in a human colonic cancer cell line in culture after treatment with sodium butyrate. *Cancer Res.*, **44**:3961-3969.
29. Huet, C., Sahuquillo-Merino, C., Coudrier, E., and Louvard, D. (1987) Absorptive mucus-secreting subclones isolated from a multipotent intestinal cell line (HT-29) provide new models for cell polarity and terminal differentiation. *J. Cell. Biol.*, **105**:345-357.
30. Gespach, C., Emami, S., and Chastre, E. (1988) Membrane receptors in the gastrointestinal tract. *Biosci. Repts.*, **8**:199-233.
31. Raymond, M., Rose, E., Housman, D. E., and Gros, P. (1990) Physical mapping, amplification, and overexpression of the mouse *mdr* gene family in multidrug-resistant cells. *Mol. Cell. Biol.*, **10**:16842-1651.
32. Mickisch, G. H., Merlino, G. T., Galski, H., Gottesman, M. M., and Pastan, I. (1991) Transgenic mice that express the human multidrug-resistance gene in bone marrow enable a rapid identification of agents that reverse drug resistance. *Proc. Natl. Acad. Sci. USA*, **88**:547-551.
33. Hammond, D. K., and Strobel, H. W. (1990) Human colon tumor cell line LS174T drug metabolizing system. *Mol. Cell. Endocrinol.* **93**:95-105.
34. Pelletier, H., Millot, J.-M., Chauffert, B., Manfait, M., Genne, P., and Martin, F. (1990) Mechanisms of resistance of confluent human and rat colon cancer cells to anthracyclines: Alterations of drug passive diffusion. *Cancer Res.* **50**:6626-6631.
35. Lesuffleur, T., Kornowski, A., Luccioni, C., Muleris, M., Barbat, A., Beaumatin, J., Dussaulx, E., Dutrillaux, B. and Zweibaum, A. (1991) Adaptation to 5-fluorouracil of the heterogeneous human colon tumor cell line HT-29 results in the selection of cells committed to differentiation. *Int. J. Cancer*, **49**:721-730.
36. Lesuffleur, T., Barbat, A., Luccioni, C., Beaumatin, J., Clair, M., Kornowski, A., Dussaulx, E., Dutrillaux, B. and Zweibaum, A. (1991) Dihydrofolate reductase gene amplification-associated shift of differentiation in methotrexate-adapted HT-29 cells. *J. Cell. Biol.*, **115**:1409-1418.
37. Jori, G., Beltrami, M., Reddi, E., Salvato, B., Pagnan, A., Ziro, L., Tomio, L., and Tsanov, T. (1984) Evidence for a major role of lipoproteins as hematoporphyrin carriers in vivo. *Cancer Lett.*, **24**:291-297.
38. Reyftmann, J. P., Morlière, P., Goldstein, S., Santus, R., Dubertret, L., and Lagrange, D. (1984) Interaction of human low density lipoproteins: a spectroscopic and photochemical study. *Photochem. Photobiol.*, **40**:721-729.
39. Candide, C., Morlière, P., Mazière, J. C., Goldstein, S., Santus, R., Dubertret, L., Reyftmann, J. P., and Polonovski, J. (1986) In vitro interaction of the photoactive anticancer porphyrin derivative photofrin II with low density lipoprotein and its delivery to cultured human fibroblasts. *FEBS Lett.*, **207**:133-138.

40. Morlière, P., Kohen, E., Reyftmann, J. P., Santus, R., Kohen, C., Mazière, J. C., Goldstein, S., Mangel, W. F. and Dubertret, L. (1987) Photosensitization by porphyrins delivered to L cells by human serum low density lipoproteins. A microspectrofluorimetric study. *Photochem. Photobiol.*, **46**:183–191.
41. Candide, C., Mazière, J. C., Santus, R., Mazière, C., Morlière, P., Reyftmann, J. P., and Dubertret, L. (1989) Photosensitization of Wi26 VA4 transformed human fibroblasts by low density lipoprotein loaded with the anticancer porphyrin mixture Photofrin II; evidence for endoplasmic reticulum alteration. *Cancer Lett.*, **44**:157–161.