Defective Internalization of Low Density Lipoprotein in Epidermoid Cervical Cancer Cells

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ABSTRACT Cells of an epidermoid cancer cell line of human uterine cervix, which possessed a high-affinity, specific receptor for low density lipoprotein (LDL), internalized and degraded [125I]iodo-LDL at a very low rate. In these cells, LDL did not stimulate cholesteryl ester synthesis, nor did it suppress 3-hydroxy-3-methylglutaryl coenzyme A reductase to the same extent as in the control cells. The binding of [125I]iodo-LDL by these cells was not decreased by preincubation of the cells in medium containing LDL. Using ferritin-labeled LDL (F-LDL) and electron microscopy, it was determined that at 4°C the cells bound F-LDL in the same way as other cancer cell lines that did not have a defect in internalization. When these cells were warmed to 37°C the F-LDL remained on the surface, whereas in cells from control cancer cell lines the F-LDL was internalized and was no longer observed on the cell surface. On the basis of the results of these studies it is concluded that cells of this epidermoid cancer cell line have a defective ability to internalize LDL.

The currently accepted model of the cellular uptake and metabolism of low density lipoprotein (LDL) was developed from the studies of Brown and Goldstein (1, 2). The processes involved in the metabolism of this lipoprotein include the initial binding of the LDL macromolecule to high-affinity, low-capacity receptors that are clustered in specialized regions on the cell surface known as coated pits (3, 4). After LDL is bound to the cell surface, the coated pits invaginate by a process resembling phagocytosis to form endocytic vesicles. These endocytic vesicles fuse with lysosomes, and the internalized LDL is hydrolyzed by lysosomal enzymes to its basic constituents: cholesterol, fatty acids, and amino acids.

The cholesterol that is liberated during lysosomal degradation of LDL may be used for cell membrane synthesis or else stored in the form of cholesteryl esters as lipid droplets (5, 6). Intracellular free cholesterol is believed to mediate the following cellular functions: (a) suppression of synthesis of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the rate limiting enzyme in de novo cholesterol biosynthesis; (b) stimulation of the activity of acyl CoA:cholesterol acyltransferase (ACAT), the enzyme that catalyzes the reaction leading to the esterification of cholesterol; and (c) down-regulation of LDL receptor synthesis.

Cancer cells replicate continuously and therefore require large quantities of cholesterol for cell membrane synthesis. The cholesterol used by cancer cells for this purpose could be derived from de novo synthesis from two-carbon molecules, or else assimilated from plasma through the uptake and degradation of LDL. It has been proposed that the control mechanisms by which cells regulate lipoprotein metabolism might be absent in neoplastic cells (7, 8). We have investigated LDL and cholesterol metabolism in several human Müllerian duct cancer cell lines (9, 10, 11) and determined that the neoplastic cells internalize and degrade LDL in the same way as other human cells (e.g. skin fibroblasts). We found that in some cases neoplastic cells metabolized LDL at a higher rate than non-neoplastic cells, with the activity being maximum during the logarithmic phase of cell growth (9, 10). Recently, Anderson et al. (12) described LDL metabolism in a human epithelioid cancer cell line (A-431). These cells bind ferritin-labeled LDL (F-LDL) in both coated and noncoated regions of the cell surface, unlike skin fibroblasts which bind F-LDL primarily in the coated regions of the cell membrane. The A-431 cells bind and degrade LDL normally although a smaller proportion of the surface-bound LDL is internalized and degraded than in skin fibroblasts or another control cell line (Chinese hamster ovary cells). The authors attributed the inefficiency of LDL internalization and degradation in the A-431 cells to the atypical distribution of LDL receptors on the cell surface.

The homozygous form of familial hypercholesterolemia is
characterized most commonly by a lack of LDL receptors on the cell surface (13). However, in one person with this disorder (J.D. [14]), the primary lesion was found to be a failure of internalization of the LDL-receptor complexes even though LDL receptors were found to be present on the cell surface of fibroblasts obtained from a skin biopsy of this individual. While studying cholesterol metabolism in several human Mullerian duct cancer cell lines, we discovered a cervical epidermoid cancer cell line (EC-168) with a defect in LDL metabolism. The results of biochemical and morphological studies presented here have led us to conclude that LDL receptors are present on the surface of EC-168 cancer cells but that the process of internalization of LDL in such cells appears to be defective.

MATERIALS AND METHODS

Materials

Culture media and fetal calf serum were obtained from Gibco (Grand Island Biological Co., Grand Island, NY). All culture flasks were purchased from Corning Glass Works (Corning, NY). The following isotopically labeled compounds were purchased from New England Nuclear (Boston, MA): [1-3H]-cholate, 3-14C-hydroxy-3-methylglutaryl coenzyme A; [1-14C] cholanic acid, and [7-3H]-cholesterol. Polyoxylethylene 10-oleylether (Brij 96), NADP+, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Co. (St. Louis, MO); [35S]-sodium iodide was purchased from Amersham-Searle (Arlington Heights, IL).

Supplies for electron microscopy were obtained from Ladd (Burlington, VT).

Ferritin-labeled LDL (F-LDL) was prepared by the method of Kishida et al. (15) as adapted by Goldstein and Brown. The specific activity of [125I]iodo-LDL was 300-500 cpm x ng-1 LDL protein.

Cells

Keratinizing, well differentiated epidermoid cervical cancer cells (EC-168) from passages 52 thru 78, and well differentiated endometrial adenocarcinoma cells (HEC-B-296) that were characterized in the past (17), were used in these studies. The EC-168 cells were derived from primary cultures of minced neoplastic tissues and were passed whenever they became confluent. The histological characteristics of these cells were confirmed by periodic evaluation of tumors grown in nude mice. The cell lines were maintained in T75 flasks in the presence of Waymouth's MB 752/1 medium containing fetal calf serum (FCS), 10% (vol/vol) and antibiotic-antimycotic mixture (1%). All culture flasks were purchased from Biological Co., Grand Island, NY. All culture flasks were purchased from Biological Co., Grand Island, NY.

Human LDL (density 1.019-1.063 g x ml-1) and lipoprotein-poor serum (LPPS) (density > 1.215 g x ml-1) were prepared by differential ultracentrifugation of plasma obtained from healthy subjects; [125I]-iodo-LDL was prepared according to the method described by Bilbeimer et al. (15) as adapted by Goldstein and Brown. The specific activity of [125I]iodo-LDL was 300-500 cpm x ng-1 LDL protein.

Determination of Specific Binding Sites for LDL

To ascertain whether the binding of LDL to the cells was specific, cells were incubated at 4°C with various concentrations of [125I]iodo-LDL in the presence or absence of a 50-fold excess of nonradioiodinated LDL. After 12 h the medium was removed, the cells were washed as described above, and the cellular uptake of [125I]iodo-LDL was measured by assay of radioactivity in homogenates prepared from washed cells. The cells were homogenized in 0.5 N NaOH.

Determination of Surface Binding, Internalization and Degradation of LDL

The cultured cells were cooled rapidly to 4°C and the medium was replaced with ice-cold medium containing LPPS. [125I]iodo-LDL was added to achieve a concentration of 10 μg LDL protein x ml-1 and the cells were allowed to stand for 2 h at 4°C. Thereafter, the medium was removed, the cells were washed five times with ice-cold, fresh medium containing LPPS (10%) and 10 μg LDL protein x ml-1 of nonradioiodinated LDL was added. The cells were then incubated at 37°C. After various times the medium was removed and assayed for content of radiolabeled degradation products of [125I]iodo-LDL. To determine surface binding, the cells were washed with ice-cold Tris-NaCl buffer (50 mM Tris, 0.15 M NaCl, pH 7.4) and then incubated in 2 ml of ice-cold buffer (pH 7.4) containing NaCl (50 mM), HEPES (10 mM) and heparin (10 μg x ml-1) for 1 h at 4°C. Thereafter, the solution was removed and an aliquot (1 ml) was assayed for radioactivity. Heparin-releasable [125I]iodo-LDL represents surface-bound material. To determine the amount of internalized radiolabeled LDL, the cells were washed again with Tris-NaCl buffer (pH 7.4) and homogenized in 1 ml of 0.5 N NaOH. An aliquot (50 μl) was used to measure cellular protein according to the method of Lowry et al. (18).

Assay of HMG CoA Reductase Activity

The cultured cells were washed twice with Tris-NaCl buffer (pH 7.4), scraped from the dishes with a rubber policeman into Tris-NaCl buffer, and pelleted by centrifugation at 1,000 rpm for 5 min. The cells were washed once by resuspension in Tris-NaCl buffer and then centrifuged. The supernatant fluid was decanted and the cell pellets were stored under liquid N2. Detergent-solubilized extracts of the cells were prepared and enzyme activity was measured according to the method described by Brown et al. (19) as adapted by Simpson et al. (20), except that 1.25% Brij 96 detergent was used rather than 0.25% KyroEOB (11). HMG CoA reductase activity is expressed as picomoles of [14C]mevalonate formed x 10-2 mg-1 cell protein x h-1.

Incorporation of [14C]Oleate into Cholesteryl Esters

After 48 h in the presence of medium containing LPPS [10% (vol/vol)], LDL, in various concentrations, was added to the media for 24 h. Thereafter, albumin-bound [14C]oleic acid (21) was added to the medium of the cultured cells to achieve a concentration of oleic acid of 150 μM and 6 h later the medium was removed, and the cells were washed with Tris-NaCl buffer (pH 7.4), harvested, and extracted with chloroform:methanol (2:1). The cholesteryl [14C]oleate was isolated by thin-layer chromatography and quantified as described previously (22). The rate of [14C]oleate incorporation into cholesteryl esters is expressed as nanomoles x mg-1 cell protein x h-1.

Electron Microscopy of Ferritin-labeled LDL

Ferritin-labeled LDL (F-LDL) was prepared by the method of Kishida et al. (23), as modified by Anderson et al. (3). After growing in culture medium containing FCS for 24 h, cells were placed in culture medium containing LPPS (10%) for 48 h. The cells were chilled to 4°C, and F-LDL (33 μg LDL protein x ml-1) was added in the presence or absence of unlabeled LDL (500 μg LDL protein x ml-1). After 2 h, the media were removed and the cells were rinsed five times with sodium phosphate buffer (0.1 M, pH 7.4). Cells were then fixed (sterilized solution) or warmed to 37°C for various periods of time in medium containing LPPS and no F-LDL. The cells were then fixed in the dishes with Na-cacodylate buffer (0.1 M) containing 1% glutaraldehyde, osmiumtetroxide, dehydrated in alcohol, and embedded in Epon 812. The embedded cells were separated from the plastic culture dishes and re-embedded in fresh Epon 812. Thin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and photographed in a Philips 300 electron microscope. Micrographs were enlarged to a final magnification of 50,000 times and evaluated by two investigators. Micrographs were randomized before evaluation and were not identified until after evaluation. The number of ferritin particles and coated regions on the cell surface membrane.
RESULTS

Biochemical Studies

The rates of uptake (i.e., binding and internalization) and degradation of \([{}^{125}\text{I}]\text{iodo-LDL}\) in two cancer cell lines, HEC-B-296 and EC-168, were determined at various times during a 30-h incubation at 37°C (Fig. 1). The degradation of \([{}^{125}\text{I}]\text{iodo-LDL}\) by HEC-B-296 cells increased with increasing time of incubation at 37°C, a pattern similar to that described in fibroblasts (16). In contrast, degradation of \([{}^{125}\text{I}]\text{iodo-LDL}\) by EC-168 cells proceeded at a very low rate, <5% of the degradation rate achieved by HEC-B-296 cells. On the other hand, levels of uptake of \([{}^{125}\text{I}]\text{iodo-LDL}\) were similar for both types of cells. A steady state of uptake was achieved at 5 h in the HEC-B-296 cells and at 11 h in the EC-168 cells.

To evaluate the effect of \([{}^{125}\text{I}]\text{iodo-LDL}\) concentration on its uptake and degradation by these cells, increasing concentrations of \([{}^{125}\text{I}]\text{iodo-LDL}\) were added to the culture media. After 6 h at 37°C, all media were removed and assayed for degradation products. Cellular uptake was measured in homogenates of the washed cells. Saturation of the uptake process was achieved at a concentration of 40 \(\mu\text{g} \times \text{ml}^{-1}\) \([{}^{125}\text{I}]\text{iodo-LDL}\) for the HEC-B-296 cells and at a concentration of \(\sim 70 \mu\text{g} \times \text{ml}^{-1}\) \([{}^{125}\text{I}]\text{iodo-LDL}\) in the EC-168 cells (Fig. 2).

To ascertain whether the binding of LDL by EC-168 cells was specific, the cells were incubated with various concentrations of \([{}^{125}\text{I}]\text{iodo-LDL}\) in the presence or absence of a 50-fold excess of nonradiolabeled LDL at 4°C for 12 h. In the presence of excess nonradiolabeled LDL, >80% of the bound \([{}^{125}\text{I}]\text{iodo-LDL}\) was displaced (Fig. 3A). Saturation binding sites was achieved at a concentration of 70 \(\mu\text{g} \times \text{ml}^{-1}\) LDL protein \(\times \text{ml}^{-1}\) of \([{}^{125}\text{I}]\text{iodo-LDL}\). Transformation of the data by the method of double reciprocals yielded a straight line from which an apparent dissociation constant \((K_D)\) was calculated to be \(36.4 \mu\text{g} \times \text{ml}^{-1}\) LDL protein \(\times \text{ml}^{-1}\) (Fig. 3B). When a similar experiment was conducted using HEC-B-296 cells, the \(K_D\) was calculated to be 20 \(\mu\text{g} \times \text{ml}^{-1}\) and saturation was achieved at 40 \(\mu\text{g} \times \text{ml}^{-1}\) LDL protein \(\times \text{ml}^{-1}\) \([{}^{125}\text{I}]\text{iodo-LDL}\) (data not shown).

The results of an experiment in which surface binding, internalization and degradation of LDL were determined in both cell lines are presented in Fig. 4. In this experiment, cells were incubated with \([{}^{125}\text{I}]\text{iodo-LDL}\) (10 \(\mu\text{g} \times \text{ml}^{-1}\) LDL protein \(\times \text{ml}^{-1}\)) for 2 h at 4°C. Thereafter, the media were removed and the cells were rinsed, fresh culture medium containing nonradiolabeled LDL was added, and the cells were warmed to 37°C. After various times the culture medium was removed and assayed for content of radiolabeled degradation products of \([{}^{125}\text{I}]\text{iodo-LDL}\). The cells were treated with heparin to determine the amount of heparin-releasable \([{}^{125}\text{I}]\text{iodo-LDL}\) (i.e., surface-bound LDL) and heparin-resistant \([{}^{125}\text{I}]\text{iodo-LDL}\) (i.e., internalized LDL). In the HEC-B-296 cells, the amount of surface-bound radioactivity diminished rapidly upon incubation at 37°C. During the first hour, there was an increase in the amount of internalized LDL. Subsequently, the amount of radioactivity in this compartment declined, concomitant with
We have shown previously that in several cancer cell lines, cultured cells were incubated for 24 h in the presence of various concentrations of LDL, and then the activity of HMG CoA reductase, an important enzyme in the regulation of de novo cholesterol synthesis, was determined. In the case of the HEC-B-296 cells, the enzyme activity decreased as the concentration of LDL in the culture medium was increased (Fig. 5). Maximum suppression of HMG CoA reductase activity (95%) was achieved at a concentration of 40 μg of LDL protein × ml⁻¹, a concentration similar to that required to saturate the LDL uptake process (Fig. 2). On the other hand, HMG CoA reductase activity of EC-168 cells was affected much less by the addition of LDL to the culture medium (i.e. a 40% inhibition at 100 μg × ml⁻¹).

We have shown previously that in several cancer cell lines in monolayer culture the rate of cholesteryl ester synthesis is stimulated by increasing concentrations of LDL (24). Cells which had been incubated with different amounts of LDL for 24 h were incubated with [¹⁴C]oleic acid, and the incorporation into cholesteryl esters was determined. In HEC-B-296 cells, the rate of cholesteryl ester synthesis, as determined by the incorporation of [¹⁴C]oleic acid into cholesteryl esters, was stimulated greatly by LDL (Fig. 6). In contrast, in the EC-168 cells, cholesteryl ester synthesis was barely detectable irrespective of the presence or absence of LDL in the culture medium.

Another characteristic of cells that metabolize LDL is down-regulation of the LDL receptor population when cells are incubated in the presence of LDL. Previous incubation of HEC-B-296 cells with LDL led to the suppression of both uptake and degradation of [¹²⁵I]iodo-LDL in a concentration-dependent manner (Fig. 7). This finding is indicative of the down-regulation of the LDL receptor population by these cells. In contrast, the uptake of [¹²⁵I]iodo-LDL by EC-168 cells was unaffected by preincubation of these cells in medium containing various concentrations of LDL. Degradation of LDL in the EC-168 cells was very low and also unaffected by preincubation with LDL. The results of this experiment are supportive of the

**Figure 4** Pulse-chase study of [¹²⁵I]iodo-LDL binding, internalization and degradation in HEC-B-296 cells and EC-168 cells. Cells were incubated with [¹²⁵I]iodo-LDL (10 μg × ml⁻¹) for 2 h at 4°C, rinsed, and fresh medium containing nonradiolabeled LDL (10 μg LDL protein × ml⁻¹) was added to the dishes. The cells were then warmed to 37°C for the indicated times. At each time-point, the media were assayed for [¹²⁵I]iodo-LDL degradation products (Δ). The cells were incubated with heparin to determine the heparin-release [¹²⁵I]iodo-LDL (i.e. the surface-bound LDL) (●) and the heparin-resistant [¹²⁵I]iodo-LDL (i.e. the internalized LDL) (○). The data presented are the average values obtained using cells from duplicate dishes.

**Figure 5** Regulation of HMG CoA reductase activity by LDL in HEC-B-296 cells (●) and EC-168 cells (○). Cultured cells were maintained in the presence of varying amounts of LDL for 24 h before homogenization and assay of HMG CoA reductase activity. The data presented are the average values obtained using cells from duplicate dishes.

**Figure 6** Rate of incorporation of [¹⁴C]oleate into cholesteryl esters in HEC-B-296 cells (●) and in EC-168 (○). Cultured cells were maintained in the presence of varying amounts of LDL for 24 h before the experiment. Cells were then incubated with [¹⁴C]oleate for 6 h, homogenized, and the incorporation into cholesteryl esters was determined. The data show the average cholesteryl [¹⁴C]-oleate production in cells from duplicate dishes.

**Figure 7** Uptake and degradation of [¹²⁵I]iodo-LDL after incubation of HEC-B-296 cells (panel A) and EC-168 cells (panel B) with various concentrations of nonradiolabeled LDL. After 48 h in the presence of nonradiolabeled LDL the cells were washed and incubated with [¹²⁵I]iodo-LDL (10 μg LDL protein × ml⁻¹) for 6 h and the rates of uptake (●) and degradation (○) were determined as described in Materials and Methods. The data presented are the average of values obtained using cells from duplicate dishes.
conclusion that the LDL receptor population in EC-168 cells is not subject to down-regulation by LDL.

Morphological Studies

To further characterize the binding sites for LDL on the surface of HEC-B-296 and EC-168 cells, the cultured cells were incubated for 2 h at 4°C with F-LDL in the absence or presence of a twelvefold excess of unlabeled LDL. The binding of F-LDL to the surface of the cells was examined by electron microscopy. F-LDL was localized to the upper surface of the cell in both cell lines. The binding of F-LDL was completely abolished in both cell lines by the addition of a twelvefold excess of unlabeled LDL to the medium during the 2-h incubation at 4°C. The F-LDL was localized in both cell lines to the straight membrane as well as to microvilli and other surface projections (Fig. 8A and C). The two cell lines had a comparable number of coated regions per mm membrane (31 per mm membrane in the HEC-B-296 cells and 20 per mm membrane in the EC-168 cells). In the HEC-B-296 cells, ~50% of the

![Figure 8](image_url)
coated regions were associated with ferritin (a mean of four ferritin molecules per coated region) (Fig. 9A). In contrast, in the EC-168 cells no ferritin was associated with coated regions (Fig. 9B).

The internalization of F-LDL was also studied in both cell lines. Cells which had been previously labeled with F-LDL at 4°C were washed and warmed to 37°C for various times in media containing LPPS (10%) and no LDL. The number of ferritin molecules per mm of surface membrane was quantitated (Table I). In the HEC-B-296 cells, 90% of the F-LDL had been removed from the cell surface after 2 h at 37°C (Fig. 8B, Table I). In contrast, in the EC-168 cells, the F-LDL remained on the cell surface, even after 2 h at 37°C (Fig. 8D, Table I). We also examined unstained sections of HEC-B-296 cells and found ferritin associated with multivesicular bodies, i.e. evidence of internalization of the F-LDL (Fig. 9C). No evidence of internalization of F-LDL was found when unstained sections of the EC-168 cells were examined. The number of F-LDL molecules bound to the surface of the HEC-B-296 cells was only about half that bound to the surface of the EC-168 cells in this experiment. This is in contrast to the data in previous experiments (Figs. 1 and 2) in which the uptake of [125I]iodo-LDL by the HEC-B-296 cells was similar to that by the EC-168 cells. These differences are reflective of the fact that the data of Table I were obtained on confluent cells whereas the data of Figs. 1 and 2 were obtained on nonconfluent cells. Furthermore, in the case of the HEC-B-296 cells, uptake includes binding and internalization, whereas in the case of the EC-168 cells the uptake and the binding are equivalent.

DISCUSSION

In the present study, evidence was obtained that cells of an epidermoid cervical cancer cell line (EC-168) have a defect in the mechanism of internalization and degradation of LDL. These cells bind LDL specifically as shown by both biochemical binding studies and ultrastructural studies with F-LDL. A similar defect of this type has been described previously in skin fibroblasts of a person suffering from a form of homozygous familial hypercholesterolemia (3, 14).

Based on the results of the present investigation, it is concluded that a defect in internalization of LDL in the EC-168 cells was responsible for the failure of LDL to regulate cholesterol metabolism in these cells. These cells, although possessing specific binding sites for LDL, have defective regulatory mechanisms for (a) suppression of de novo cholesterol synthesis by LDL, (b) stimulation of cholesterol esterification by LDL, and (c) down-regulation of LDL receptors by LDL. These regulatory mechanisms were present in cells of another cancer line (HEC-B-296) in which LDL was internalized in a normal fashion. In other cancer cell lines that we have studied (9) and in a cancer cell line investigated by Anderson et al. (12),

Understanding the internalization and degradation of LDL is crucial for the study of cholesterol metabolism in cancer cells. The differences observed between the HEC-B-296 and EC-168 cells highlight the importance of these processes in cellular function. Further studies could explore the molecular mechanisms underlying these differences.
internalized LDL regulates cholesterol metabolism in the same manner as has been described for the skin fibroblast by Brown and Goldstein (2).

Anderson et al. (12) have recently described the binding and internalization of LDL in cells of a cancer line, A-431 cells, and in Chinese hamster ovary cells. In the Chinese hamster ovary cells, the F-LDL was localized primarily in coated regions, similar to the pattern described in skin fibroblasts. The A-431 cells, however, had F-LDL distributed evenly over their upper cell surface, with a relatively small percentage of the F-LDL being associated with coated areas. The pattern of F-LDL binding we observed in the HEC-B-296 and EC-168 cells was similar to that of the A-431 cells, i.e. relatively few receptors on the cell membrane were localized to the coated area. We have also studied another cervical epidermoid cancer cell line (EC-50) which was characterized in the past (25). In these cells, we observed a pattern of F-LDL binding similar to that of the HEC-B-296, EC-168 cells, and A-431 cells (data not shown). Thus, there appear to be at least two distinct patterns of LDL receptor localization, one in which the receptors are localized primarily in coated regions, as described for skin fibroblasts and Chinese hamster ovary cells, and another in which the receptors are more evenly distributed over the upper cell surface, as described in the case of three cancer cell lines, i.e. the A-431, HEC-B-296, and EC-50 cells. Both types of cells can bind, internalize, and degrade LDL and thus regulate cholesterol metabolism, but their “strategies” for accomplishing this differ. The cancer cell lines are able to bind larger quantities of LDL of which a fraction is internalized, while the fibroblasts and Chinese hamster ovary cells bind less LDL but internalize it almost completely. The abundance of LDL receptors on the surface of some cancer cells enables them to assimilate cholesterol at a high rate (9). This cholesterol is required for cell membrane synthesis in these continuously replicating cells.

Brown and Goldstein (14), who described a defect in the internalization of LDL in J. D. fibroblast cells, suggested that the LDL receptor complex is composed of at least two components, one which brings about binding of the lipoprotein and the other which facilitates the internalization of LDL once binding has occurred. The absence of the latter component would cause defective LDL metabolism in such cells by failure of LDL internalization. The defective internalization of LDL in the epidermoid cervical cancer cells (EC-168) studied in the present investigation could conceivably be of a similar nature. Although the number of coated regions per millimeter of membrane in the EC-168 cells was within the range reported by Anderson et al. for normal skin fibroblasts (3), A-431 cells, and Chinese hamster ovary cells (12), no F-LDL was observed associated with these coated areas. Thus, the defect in the EC-168 cells appears to be similar to that described in the J. D. skin fibroblasts (14). Although the receptor in EC-168 cells binds LDL specifically, the receptor-LDL complex does not associate with a coated region and the bound LDL is not internalized.

Many investigators are searching for a biochemical explanation(s) for neoplastic transformation of cells and have suggested that the neoplastic state is characterized by “lack of control” of intracellular events by extracellular signals (7, 8, 26). The defect in cholesterol metabolism in the EC-168 cells is of this type, namely failure of external factors (plasma LDL) to regulate intracellular cholesterol metabolism. These neoplastic cells with a defect in LDL internalization could provide a useful model for the investigation of the structural modifications that lead to failure of adsorptive endocytosis.

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