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Expression of the Low-Density Lipoprotein Receptor, HMG-CoA Reductase, and Multidrug Resistance (Mdr1) Genes in Colorectal Carcinomas

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ABSTRACT. Some malignant cells have elevated uptake of plasma low-density lipoprotein (LDL). We determined the expressions in colorectal cancers of the LDL receptor gene, of the gene for the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and of the multidrug resistance gene (mdr1) by quantitative RNA-RNA solution hybridisation. LDL receptor RNA levels in tumor tissue exceeded those in normal mucosa in 20 of 23 patients (2–11-fold higher in 17 of 23 patients), with a mean ± SD of 7.8 ± 5.8 copies/cell in tumor tissue vs 3.5 ± 2.5 in normal mucosa ($P = 0.002$). The HMG-CoA reductase gene was similarly expressed in tumor and normal tissue, with means and SD of 2.0 ± 1.3 copies/cell versus 2.2 ± 1.9 ($n = 21$). Mdr1 RNA was undetectable (<0.15 copies/cell) in 5 of 20 tumors, with a mean ± SD of 1.0 ± 1.1 copies/cell vs 1.6 ± 1.7 in normal mucosa. Expression of all three genes was, in most cases, higher in normal liver than in liver metastasis of colorectal carcinomas or normal colon mucosa. The results may form the basis for using LDL as a drug carrier for treatment of colorectal carcinomas, and may indicate that drug resistance in these tumors is not due to overexpression of the mdr1 gene. BIOCHEM PHARMACOL 52;1:127–131, 1996.

KEY WORDS. gene expression; RNA probes; LDL receptors; multidrug resistance; cancer

Identification and utilization of differences in metabolic processes between normal and malignant cells are essential to develop new treatment strategies for cancer. One such difference may be that of receptor-mediated uptake of LDL¶, which is the major cholesterol-carrying lipoprotein of human plasma, containing about two thirds of plasma cholesterol [1]. Cellular cholesterol requirements are met by receptor-mediated uptake of LDL and/or endogenous synthesis, the rate of the latter being limited by the enzyme HMG-CoA reductase EC 1.1.1 88 [1]. After it is bound to the cell surface LDL receptor, LDL is internalized and degraded in lysosomes and cholesterol is subsequently released to be used by the cells.

Acute myelogenous leukemia cells have elevated receptor-mediated uptake of LDL compared with white blood and nucleated bone marrow cells from healthy subjects [2, 3]. Hypocholesterolemia is frequently found in patients with acute leukemia, and cholesterol levels are inversely correlated with the LDL receptor activities of leukemic cells, suggesting that the underlying mechanism is increased catabolism of LDL by the leukemic cells [4]. Hypocholesterolemia is also associated with lung cancer [5] and elevated uptake of LDL by human lung cancer tissue *in vivo* has been demonstrated [6]. Additional support of high LDL uptake by solid tumors comes from studies in experimental animal models [7, 8].

Hypocholesterolemia has also been reported in patients with colon cancer [9], and a negative correlation observed between cholesterol levels and colon cancer mortality [10, 11]. We, therefore, studied expression of the LDL receptor gene in tumor and normal tissue from patients with colorectal cancer. The expressions of the gene for HMG-CoA reductase and the MDR gene (mdr1) [12] were also quantified because these tumors are often drug-resistant. The mdr1 gene encodes a 170 kD glycoprotein (Pgp) [13, 14] that is believed to act as a drug efflux pump because resistant cells accumulate less drug *in vitro* than sensitive cells [15]. Elevated expression of the LDL receptor gene in colorectal cancer tissue could present the possibility of targeting cytostatics by incorporating them into LDL, possibly overcoming Pgp-mediated drug resistance.

MATERIALS AND METHODS

Tissue samples

Twenty-three paired samples of primary colorectal cancers and normal mucosa were studied (Table 1). Five patients were represented by liver metastasis of colorectal cancers and/or normal liver, one of them also by the colonic tumor and mucosa (Table 2). The mucosa was dissected from the

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¶ *Abbreviations:* HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low-density lipoprotein; MDR, multidrug resistance; Pgp, P-glycoprotein; TNA, total nucleic acids.

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TABLE 1. Patient and tumor characteristics

Patient no.	Sex/age	Differentiation	Tumor cells (%)
1	M/63	I	80
2	F/62	I	60–75
3	F/49	I	60–90
4	M/84	P	40–65
5	M/61	I	40–80
6	F/85	I	50–85
7	M/77	I	35–90
8	F/79	I	65
9	F/72	I	65
10	M/57	I	45
11	M/58	P	60
12	M/74	I	40–80
13	M/65	I	45–80
14	F/63	P	65–80
15	F/81	I	60–85
16	F/84	I	65–90
17	M/72	P	65
18	F/68	I	40–65
19	M/82	W	75
20	M/59	I	35
21	F/82	I	70
22	M/81	I	85
23	M/72	P	40

All tumors were adenocarcinomas. Differentiation grade: W, well differentiated; I, intermediately differentiated; P, poorly differentiated.

submucosa, nonnecrotic tumor was selected, and samples were frozen in liquid nitrogen. Adjacent tumor was sampled for histopathology. Informed consent was obtained as required by the Ethical Committee of the Karolinska Institute.

Determination of RNA Levels

LDL receptor, HMG-CoA reductase, and mdr1 RNA levels in tissues were determined by a quantitative RNA-RNA solution hybridization method [16, 17]. Total nucleic acids (TNA) were prepared from tissue of approximately 200 mg wet weight [18] that was homogenized in 4 mL of 1 × SET (1% SDS, 20 mM Tris-HCl, pH 7.5, and 10 mM EDTA) with a Polytrone (Kinematica Type PT 16/35, Kriens, Lucerne, Switzerland) for 10–15 sec at setting 5–6; DNA concentration was assayed by Hoechst fluorometry [19]. Antisense and sense probes for LDL receptor RNA were prepared by *in vitro* transcription of a BamHI or HindIII cleaved plasmid, carrying a 265-bp fragment of cDNA encoding the human LDL receptor, with T7 (in the presence of ^{35}S-UTP and ^{35}S-CTP) and SP6 (in the presence of unlabeled nucleotides only) RNA polymerases, respectively [17]. HMG-CoA reductase RNA antisense and sense probes were prepared by *in vitro* transcription of a HindIII or EcoRI-cleaved plasmid, carrying a 50-bp oligonucleotide encoding human HMG-CoA reductase, with SP6 and T7 RNA polymerases, respectively [17]. An antisense probe for mdr1 RNA was prepared by *in vitro* transcription of a StuI cleaved plasmid, carrying the 1383 bp mdr1 cDNA sequence 5A (kindly provided by M. M. Gottesman and I. Pastan, NCI, U.S.A. [20], with SP6 RNA polymerase in the presence of ^{35}S-UTP (393 nucleotides transcribed from 5A). A sense probe was transcribed with SP6 RNA polymerase from an EcoRI-cleaved plasmid, into which the 393-bp sequence from 5A had been subcloned. The nucleic acid extracts or unlabeled sense RNA were hybridized with the various antisense probes in solution. Following RNase treatment and precipitation with trichloroacetic acid, the RNase-resistant precipitate was collected on a filter and the radioactivity determined in a liquid scintillation counter (Packard Instrument Company, Downers Grove, IL, U.S.A.). Rnase protection experiments demonstrated that the protected fragments were of the expected sizes [16, 17]. Blank values were below 0.4% of the input radioactivity. RNA levels were quantified by comparing the hybridization signal with the linear part of the standard curve, which was generated by hybridizations with different concentrations of sense RNA. All RNA values are based on 3 serial dilutions of each extract, and presented as RNA copies per diploid genome (6 pg DNA). The limit of detection, set as twice the background radioactivity, was equivalent to 0.10, 0.20, and 0.15 copies/cell in samples containing 40 μg of DNA, for the LDL receptor, HMG-CoA reductase, and mdr1 RNA assay, respectively. The coefficient of variation between identical extracts was 2.2% and 12% for the LDL receptor and HMG-CoA reductase RNA assay, respectively [17], and 25% for the mdr1 RNA assay [17].

The recovery of *in vitro* labeled RNA was checked in various tissues. Uncleaved plasmid pGEM-5Zf(+) (Promega Corporation, Madison, WI, U.S.A.) was transcribed with SP6 RNA polymerase in the presence of ^{35}S-UTP, and the *in vitro* labeled RNA was added to tissue samples during homogenization. The recoveries of RNA (high-molecular radioactivity) in the final TNA preparation from normal liver (n = 1), normal colon (n = 1), and colon tumor tissue (n = 1) were 97%, 101%, and 97%, respectively.

Statistical Analysis

Significance levels were derived from Student's t-test for paired observations.

RESULTS

LDL receptor RNA levels in tumor tissue exceeded those in normal mucosa in 20 of 23 patients (2–11-fold higher in 17 of 23 patients; Fig. 1A). For all patients, the mean and SD LDL receptor RNA levels were 7.8 ± 5.8 copies/cell for tumor tissue and 3.5 ± 2.5 copies/cell for normal tissue (P = 0.002, 95% confidence interval for difference 1.8 to 6.8 copies/cell). Expression of the HMG-CoA reductase gene was similar in tumor and normal tissue, with means and SD of 2.0 ± 1.3 copies/cell versus 2.2 ± 1.9, respectively (NS, P = 0.61, n = 21). Mdr1 RNA was undetectable (<0.15 copies/cell) in 5 of 20 tumor samples, whereas, all samples from

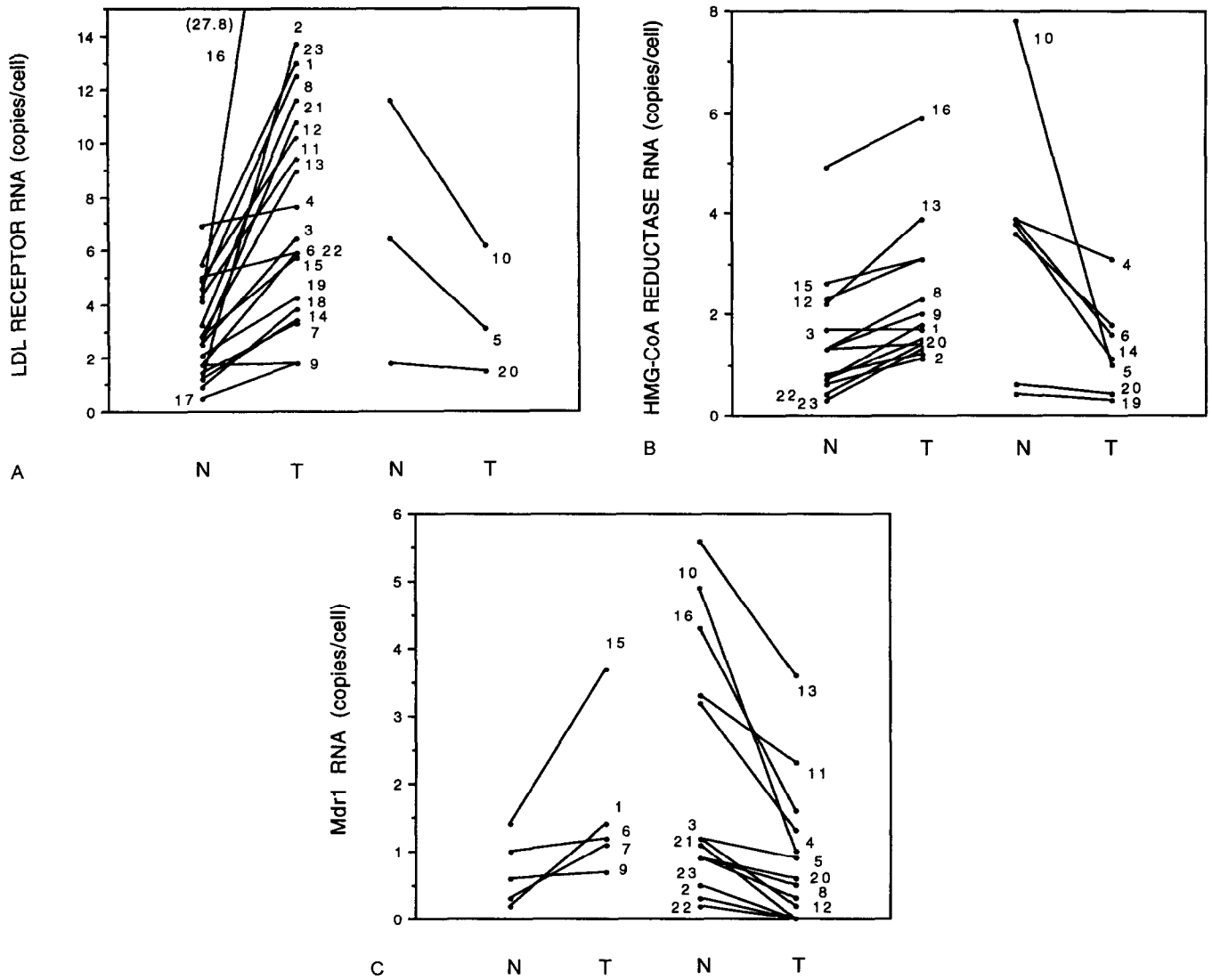

FIG. 1. Summary of (A) LDL receptor, (B) HMG-CoA reductase, and (C) mdr1 RNA levels from paired samples of normal mucosa (N) and tumor tissue (T) from patients with colon cancer. Left, cases with tumor levels ≥ normal levels; right, tumors levels < normal tissue. Patient numbers as in Table 1. Each value is the mean results of 3 hybridisations from serial dilutions of each colorectal extract.

normal mucosa had detectable levels. Mean and SD mdr1 RNA levels were 1.0 ± 1.1 and 1.6 ± 1.7 copies/cell for tumor and normal tissue, respectively (NS, $P = 0.076$, $n = 20$). (Samples with <0.15 copies/cell were set to 0.10 copies/cell). The RNA levels in tumor and normal tissue for each patient are graphically presented in Fig. 1A–C.

RNA levels for three genes in liver biopsies from 5 patients are given in Table 2. Expression of all three genes was uniformly much higher in healthy liver than in healthy colon mucosa or liver colorectal metastase. Patient No. 3 had, at primary surgery, an intermediately differentiated adenocarcinoma of the colon (Table 1), with 6.4 and 1.7 copies/cell of LDL receptor and HMG-CoA RNA, respectively. Eleven months later, a resected liver metastasis had increased the RNA levels for the LDL receptor and HMG-CoA reductase genes to 10.1 and 8.1 copies/cell, respectively. However, expression of the LDL receptor gene in normal liver tissue was higher (25.5 copies/cell) than in the metastasis. Mdr1 RNA levels in the primary tumor and the liver metastasis were very low (0.2 and 0.6 copies/cell, respectively), whereas normal liver tissue had higher mdr1 gene expression (4.1 copies/cell).

DISCUSSION

We found that colorectal cancers usually had higher LDL receptor gene expression than normal colon mucosa. HMG-CoA reductase gene expression was similar in both tissues, indicating that the cancer cells preferentially meet their increased cholesterol requirements by increased LDL receptor activity and not by elevated cholesterol synthesis. Previously, ^{125}LDL binding to homogenates of various human tissues was assayed and the only colon cancer had

TABLE 2. Patient characteristics and individual RNA levels for normal liver and liver metastasis tissue from 5 patients with metastatic colon cancer

Patient no.	Sex/age	Tissue/ (differentiation) % tumor cells	RNA copies/cell		
			LDL rec	HMG-CoAr	Mdr1
24	M/77	Normal liver	26.1	13.1	5.9
		Liver met.* (I), 85%	6.9	2.6	1.3
25	F/45	Normal liver	43.9	11.2	5.7
26	M/42	Normal liver	9.3	2.9	6.1
27	F/37	Normal liver	3.0	1.3	n.d.
3	F/49	Normal liver	25.5	4.1	4.1
		Liver met. (I), 90%	10.1	8.1	0.6

* met., metastasis.
Each value is the mean result of 3 hybridisations. Differentiation grade: as in Table 1.

approximately 2–4-fold higher binding than normal colon tissue [21], which agrees with our results. No other data have previously been reported on LDL receptor expression in colon cancer. A matched case-control study found significantly lower serum cholesterol levels in colon cancer patients with advanced tumors than in healthy controls [9]. The assumption was that low serum cholesterol levels in such patients may be a result of the metabolic influence of advanced tumors. Our findings suggest that this influence is mediated by increased LDL receptor gene expression.

LDL receptor gene expression was high in liver. Liver LDL receptor activity has a central role in controlling plasma cholesterol levels [1]. Few studies have, however, compared LDL receptor expression between different tissues and organs in humans. The current findings are in agreement with previous results [21] that showed higher binding of ^{125}LDL to homogenates of liver than of colon. Also, the *in vivo* uptake of ^{14}C-sucrose-LDL, following i.v. injection, was approximately 25% higher in liver than in colon in a patient with acute leukemia [22]. Interestingly, only the adrenals and the liver had a higher uptake than the colon, indicating that colorectal cancers may have higher LDL receptor expression than most other organs.

Measurements of gene expression at the RNA level may not necessarily reflect protein content or protein activity. Indeed, multilevel and multivalent regulation of the HMG-CoA reductase gene has been established previously [23], and has been suggested for LDL receptor gene expression as well [17]. However, in the latter study, LDL receptor and HMG-CoA reductase RNA levels correlated overall with corresponding protein activities. For the mdr1 gene, expression at the RNA level correlated with drug resistance in cell lines *in vitro* [24–26]. In highly resistant cells, protein levels correlated better with resistance than did mdr1 RNA [27]. Discrepant RNA and protein levels were also noted in clinical samples, suggesting that expression is not only regulated at the RNA level [28, 29].

Using a sensitive assay method, we found that mdr1 RNA levels in colorectal tumors were generally low compared to that in normal tissue. Others have shown that the adrenals and kidneys had high mdr1 RNA levels, whereas intermediate levels were expressed in lung, liver, jejunum, colon, and rectum, and low levels in other tissues [30]. Tumors of the adrenals, kidney, colon, and liver often had high levels of mdr1 RNA [30, 31]. Comparisons were made with normal colon tissue [30]. As in our study, mdr1 RNA levels in healthy tissue were mostly equal to or higher than those in tumors. Mdr1 RNA levels in normal colorectal and gastric tissues adjacent to tumors were also similar to those in the tumors in another study [32], as was the content of membrane P-glycoprotein in colorectal tumors and adjacent normal tissue [33]. The low expression of the mdr1 gene in colorectal cancer tissue suggests that drug resistance in these tumors is not caused by P-glycoprotein-mediated drug transport. Recently, it was shown that both relapse-free survival and overall survival did not differ between patients with mdr1 RNA-positive and negative tumors [34]. The lack of effect of combining MDR reversal agents with vinblastine or epidoxorubicin in the treatment of colorectal cancer also supports the conclusion that other mechanisms than MDR are important [35, 36].

Elevated LDL receptor expression in colorectal cancer tissue could suggest the possibility of administering anticancer agents incorporated into LDL particles to patients with these malignancies. Cytotoxic drugs can be incorporated into LDL particles and delivered to cells *in vitro* and *in vivo* [37]. The adrenals and the liver, which have high LDL uptake, may suffer from the cytotoxic effect of such drug-LDL complexes. It may, however, be possible to circumvent this problem by pretreatment with steroids and bile acids [7].

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