COORDINATE UP-REGULATION OF LOW-DENSITY LIPOPROTEIN RECEPTOR AND CYCLO-OXYGENASE-2 GENE EXPRESSION IN HUMAN COLORECTAL CELLS AND IN COLORECTAL ADENOCARCINOMA BIOPSIES

Donald F. Lumm1,2, Kenneth R. McQuaid1,2, Vicki L. Gilbertson2,3 and Millie Hughes-Fulford1,2,*

1Department of Medicine, Veterans’ Affairs Medical Center, San Francisco, CA, USA
2Department of Medicine, University of California, San Francisco, CA, USA
3Laboratory of Cell Growth, University of California and VA Medical Center, San Francisco, CA, USA

Many colorectal cancers have high levels of cyclo-oxygenase 2 (COX-2), an enzyme that metabolizes the essential fatty acids into prostaglandins. Since the low-density lipoprotein receptor (LDLr) is involved in the uptake of essential fatty acids, we studied the effect of LDL on growth and gene regulation in colorectal cancer cells. DiFi cells grown in lipoprotein-deficient sera (LPDS) grew more slowly than cells with LDL. LDLr antibody caused significant inhibition of tumor cell growth but did not affect controls. In addition, LDL uptake did not change in the presence of excess LDL, suggesting that idlr mRNA lacks normal feedback regulation in some colorectal cancers. Analysis of the idlr mRNA showed that excess LDL in the medium did not cause down-regulation of the message even after 24 hr. The second portion of the study examined the mRNA expression of idlr and its co-regulation with cox-2 in normal and tumor specimens from patients with colorectal adenocarcinomas. The ratio of tumour-derived normal mucosa of mRNA expression of idlr and of cox-2 was measured in specimens taken during colonoscopy. idlr and cox-2 transcripts were apparent in 11 of 11 carcinomas. There was significant coordinate up-regulation both of idlr and of cox-2 in 6 of 11 (55%) tumors compared with normal colonic mucosa. There was no up-regulation of cox-2 without concomitant up-regulation of idlr. These data suggest that the LDLr is abnormally regulated in some colorectal tumors and may play a role in the up-regulation of cox-2. Int. J. Cancer 83:162–166, 1999.

Cancer of the colon is one of the most common cancers in Western countries, including North America, showing a more than 10-fold excess over rates in Asia, Africa and parts of South America. Colon cancer is the 2nd leading cause of malignancy deaths in the United States, with approximately 150,000 new cases and 56,000 deaths yearly (Wingo et al., 1995).

Although the specific cause of colon cancer is unknown, epidemiological studies suggest that dietary factors are important in its development in humans (Yeung et al., 1991). These studies have revealed that diets high in total fat and low in dietary fibers are associated with increased risk of developing colon cancer. Furthermore, dietary fat itself may be a risk factor for colon cancer irrespective of dietary fiber content. This positive association between dietary fats and colon cancer occurs in diets especially high in polyunsaturated fats. Animal studies confirm the tumor-promoting effects of dietary fats on colon cancer (Reddy, 1992). These studies have shown that specific types of polyunsaturated fat have a greater tumor-promoting effect (Reddy, 1992). Colon cancers have high levels of fatty acids or their products stored in cell membranes, suggesting a role for fatty acids in carcinogenesis (Awad et al., 1993; Nicholson et al., 1991). The essential fatty acid linoleic acid is converted to arachidonic acid, a 20-carbon fatty acid. Arachidonic acid is further biosynthesized into various prostaglandins. The arachidonic acid cascade generates a group of bioactive lipids that modulate various physiological and pathophysiological events. There is much interest in the potential involvement of various arachidonic acid metabolites in the genesis of colorectal carcinoma. As mentioned, linoleic acid is the precursor of arachidonic acid. The low-density lipoprotein receptor (LDLr) regulates the majority of essential fatty acid uptake as well as cholesterol uptake into cells. The essential polyunsaturated fatty acids are then esterified to phospholipids. Once arachidonic acid is released from phospholipids, it is oxidized by 1 of 3 oxygenases: cytochrome P-450, lipoxigenase and cyclo-oxygenase 1 (COX-1) or COX-2. The COX pathway leads to production of various prostaglandins, including PGE2. LDLr play a key regulatory role in the initial uptake of essential fatty acid into cells and the subsequent synthesis of eicosanoids such as PGE2 (Habenicht et al., 1990, 1992). In sum, epidemiological and experimental data strongly implicate fatty acids as an important factor in the development of colon cancer. COX-2, an enzyme that metabolizes the essential fatty acids into prostaglandins, was first noted to be up-regulated in colorectal cancer by Eberhart et al. (1994). Since then, there have been numerous reports of over-expression of cox-2 in colorectal and prostate tumors and chemoprevention by COX inhibitors (Thun et al., 1991; Eberhart et al., 1994; Kargman et al. 1995; Tjandraawinata and Hughes-Fulford, 1997; Tjandraawinata et al., 1997).

Certain tumors, such as colorectal carcinoma and human adenocarcinoma cell lines, have high levels of LDLr protein (Caruso et al., 1993; Gueddari et al., 1993). We have examined mRNA expression of idlr and cox-2 in cell lines as well as in normal and tumor biopsies from patients with colorectal adenomas and adenocarcinomas. The data demonstrate a loss of feedback regulation of the LDLr. In addition, we show here that cox-2 is up-regulated in tumors that have over-expressed idlr mRNA compared with normal mucosa. Since COX-2 is known to be a feed-forward enzyme, i.e., the message is up-regulated by its substrates/products, it is possible that cox-2 message is up-regulated because some tumors have increased idlr mRNA. Since high levels of cox-2 mRNA, protein or eicosanoid products in tumors have been observed by other investigators, we postulated that human colon cancers with high levels of cox-2, cellular arachidonic acid or eicosanoids may respond to inappropriate uptake of essential fatty acids through LDLr. The current study suggests that idlr is abnormally regulated in some tumors and may play a role in the up-regulation of cox-2 in these tumors.

MATERIAL AND METHODS

Material

LDL isolated from human plasma was purchased from Sigma (St. Louis, MO). Fluorescent Dil-LDL probe was purchased from Molecular Probes (Eugene, OR). Leibovitz’s L-15 medium, DMEM, L-glutamine and trypsin were purchased from the UCSF Cell

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*Correspondence to: Laboratory of Cell Growth (151F), University of California, San Francisco and Department of Veterans’ Affairs Medical Center, 4150 Clement Street, San Francisco, CA 94121, USA. Fax: +1-415-476-1267, E-mail: milliehf@AOL.com

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Culture Facility (San Francisco, CA). FBS was from HyClone (Logan, UT). Human lipoprotein-deficient serum (LPDS) and antibiotic-antimycotic solution (containing penicillin, streptomycin and amphotericin B) were from Sigma Cell Culture. The LDLr blocking antibody was a generous gift from Dr. M. Brown, Southwestern Medical School, University of Texas Health Science Center (Dallas, TX).

Cell culture
The DiFi colorectal cancer cell line was developed by Dr. B. Boman, who derived the cells from a familial adenomatous polyposis (FAP) patient with Gardner’s syndrome characteristics (Gross et al., 1991). Cells were grown in an incubator at 37°C with 5% CO2 in 10% FBS in a 1:1 mix of Leibovitz’s L-15 medium and DMEM (H-16 with 1 g/l glucose). Medium was supplemented with insulin/transferrin/Na selenite and an antibiotic-antimycotic solution (both from Sigma) as well as L-glutamine. Cells were maintained at high density in a 37°C incubator with 5% CO2 and fed 3 times a week.

Measurement of Dil LDL uptake
Cell stocks were fed with fresh 10% FBS-containing medium 24 hr before plating. Cells were plated in 96-well plates at a density of 1.5 × 105 cells/well and grown in 2% LPDS-containing medium for 24 hr; 5 μl LDL/ml medium were then added to the LDL-positive samples. Cells were then incubated for 24 hr, to up-regulate the LDLr. To determine relative LDL receptor protein after the incubation period, Dil-LDL (2 μg/ml) was added to the medium. Samples lacking exogenous LDL were incubated with equal final molar concentrations of cold LDL during the 1.5 hr labeling, to keep the DiFi-LDL/LDL molar concentration equivalent. Cells were then rinsed 4 times with PBS, and Dil-LDL uptake was quantified with a Fluoroskan II fluorometer (Labsystems; Needham Heights, MA) based on the method described by Stephon and Yuracheck (1993). Fluorescence was read with the excitation wavelength set at 520 nm and the emission at 578 nm. Parallel samples were plated on sterile coverslips, following the same protocol, and labeled with Dil-LDL. Coverslips were kept moist with PBS and inverted onto glass microscope slides. Slides were viewed and photographed with a Zeiss Axioscope microscope at 40× magnification.

DiFi cell growth in the presence of LDLr blocking antibody
This method has been described by Habenicht et al. (1990). Cells were grown either in 1% FCS ± LDLr antibody or 10% LPDS ± 8 μg LDL or ± LDL antibody for 48 hr before analysis of cell number.

Tissue procurement and clinical history
The study was approved by the Committee on Human Research. In all, 12 colorectal carcinoma samples and 2 adenoma samples were obtained at the time of colonoscopy by means of jumbo biopsy forceps. Biopsies of accompanying normal mucosa were collected for comparison in all but 1 case, which, although it had increased cox-2 expression, was not used in data calculations. All tissues were placed in 1.5-ml siliconized microfuge tubes and stored at 70°C. Patients were asked whether they were regular users of non-steroidal anti-inflammatory drugs (NSAIDs). A serum lipid profile, when available, was obtained from the patients’ medical records.

RNA isolation
RNA STAT-60 reagent (1 ml) was added directly to the single biopsy tissue in the 1.5-ml siliconized microfuge tube. The tissue was gently pulverized by hand with a plastic agar. Total RNA was isolated by the acid guanidinium-thiocyanate/acid phenol/chloroform extraction method (RNA STAT-60 reagent) according to the procedure recommended by the manufacturer (TelTest, Friendswood, TX). The resulting RNA was dissolved in diethylpyrocarbonate (DEPC)-treated water and run on a 1% agarose gel. RNA was quantitated on a GeneQuant spectrophotometer (Pharmacia LKB, Piscataway, NJ) and the purity confirmed by 28S and 18S bands on the RNA gel.

RT-PCR analysis
RNA (1.5 μg) was reverse-transcribed in the presence of 1 mM each of deoxynucleotide (Boehringer-Mannheim, Indianapolis, IN), 2.5 μM oligo (dT)12-18 primer (GIBCO-BRL, Gaithersburg, MD), 600 U Rnase inhibitor (Boehringer-Mannheim), 300 U M-MLV reverse transcriptase (GIBCO-BRL) and 5× first-strand buffer supplied together with the M-MLV reverse-transcriptase enzyme. Reverse transcription (RT) was carried out in a Robocycler 40 Temperature Cycle (Stratagene, San Diego, CA) with a hybridization step at 30°C for 10 min, reverse transcription at 42°C for 42 min, de-naturation at 99°C for 5 min and cooling down at 6°C for 5 min. The reverse transcriptase reaction was then divided into separate PCRs to analyze and compare genes of interest. Samples were analyzed in the linear portion of the PCR amplification. The PCR reaction was carried out in an total volume of 50 μl containing 5 μl of single-stranded cDNA from RT sample, 2.5 mM MgCl2 (GIBCO-BRL), 1 mM of each deoxynucleotide (Boehringer-Mannheim), 2.5 U Taq DNA polymerase (GIBCO-BRL), 10× PCR buffer supplied with the Taq DNA polymerase, 25 pmol of each forward and reverse gene primer and sufficient de-ionized water to make up the 50-μl total volume. Oligonucleotide primers were designed to span at least 1 intron, to detect any contaminating genomic DNA. Primers used for priming the ldlr gene were as follows: sense, 5’ to 3’, CAATGTCTCCCAAAGCTT, anti-sense, 5’ to 3’, TCTGTCTCAGGGGTAGCAT. Primers used for cox-2 were as follows: sense, 5’ to 3’, CCCCATACCCCGC-CAAAGG, anti-sense, 5’ to 3’, AAAATAGGGAAAC-GGAATGT. Primers used for the internal standard cyclophilin were as follows: sense, 5’ to 3’, CACTTCCTTTAGCTTACCCAC, anti-sense, 5’ to 3’, CATATCAATTTAATTCTCT-CATACCC. Each cycle was carried out at 94°C for 1 min 40 sec, 63°C for 1 min 10 sec and 72°C for 1 min 40 sec. The RNA content was held constant; linear RT-PCR was accomplished by varying the number of PCR cycles. PT conditions were established so that the RNA was not limiting and the PCR amplification was stopped in the linear range where reaction products could be accurately quantified and compared. PCR bands were identified by size after electrophoresis on a 2% agarose gel, stained with etidium bromide, and photographed with a Polaroid camera over UV light. For quantification, the bands of interest were scanned at 400 dpi (Scanjet 4c; Hewlett-Packard, Palo Alto, CA) and stored as Macintosh TIFF files. Peak areas and densities were determined using NIH Image 1.5 matching software (NIH, Bethesda, MD).

RESULTS
Loss of feedback regulation of LDLr protein and message in colorectal cancer cells
To determine whether LDLr is normally regulated in colorectal cancer cells, DiFi cells were grown in 2% LPDS for 24 hr with and without LDL. After incubation with 6 μg/ml of LDL for 24 hr, cells were analyzed for total amount of LDL uptake (Fig. 1. Table I) and ldlr mRNA (Fig. 2). LPDS did not up-regulate the LDLr message or uptake of lipoprotein, and addition of exogenous 6 μg LDL/ml of medium did not down-regulate the functional protein (as measured by Dil-LDL uptake) or mRNA expression in these tumor cells. Feedback regulation of the LDLr was not present in the DiFi cell line. Normal regulation was seen in fibroblasts (data not shown).

LDLr is required for growth
As shown in Figure 3, cells grown in 10% LPDS without LDL were essentially as quiescent as cells in 1% FCS. When exogenous LDL was added, the cell number practically doubled over 48 hr, which is the normal growth rate. When LDLr blocking antibody was added to cells with LDLr growth was significantly reduced, p = 0.001) (Fig. 3). Addition of the antibody to the slowly growing controls did not have any significant effect on cell growth or health.
FIGURE 1 – LDLr-mediated uptake of Dil-labeled LDL in DiFi cells. LDL uptake is seen in the bright areas. Normal regulation of the LDLr would be seen by a reduced uptake of LDL. Cells were grown for 24 hr in 2% LPDS before medium was supplemented with LDL. Cells were then grown in 2% LPDS (a) without LDL or (b) with LDL (6 μg/ml) for 24 hr before addition of Dil-LDL. Cold LDL (6 μg/ml) was added to the samples lacking LDL during the 24-hr incubation with the Dil-LDL fluorescent label (90 min) to keep the Dil-LDL:LDL molar concentration equivalent.

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Relative fluorescence per well</th>
</tr>
</thead>
<tbody>
<tr>
<td>−LDL</td>
<td>5.9 ± 1.2</td>
</tr>
<tr>
<td>+LDL</td>
<td>6.8 ± 2.3</td>
</tr>
</tbody>
</table>

DiFi cells were grown in LPDS as described in Material and Methods using 96-well plates. Read-out was corrected for cell number (n = 8).

FIGURE 2 – ldlr mRNA is not regulated by LDL in DiFi cells. Representative expression of the ldlr message in DiFi cells visualized on an ethidium bromide gel. Cells were grown as described above, then incubated with LDL for (1) 0 hr, (2) 1 hr, (3) 2 hr, (4) 6 hr, (5) 12 hr or (6) 24 hr. mRNA was determined by RT-PCR as described in Material and Methods.

suggesting that LDLr is essential for growth of these colorectal cancer cells.

Abnormal regulation of LDLr in tumor biopsies

A total of 11 cases of paired sporadic adenocarcinoma and normal mucosa from either surgical resection or colonoscopy were evaluated (Table II). Equal amounts of RNA were verified by examination of the RNA gel, quantification by UV absorbance and finally by analysis of RT-PCR bands of the internal standard cyclophilin on an ethidium bromide–stained gel. Low levels of cox-2 mRNA and ldlr mRNA were observed in each of the normal mucosa samples. cox-2 and ldlr transcripts were apparent in 11 of 11 carcinomas. The degree of up-regulation of cox-2 and ldlr mRNA in the carcinoma compared with normal control, as determined by relative pixel densitometry, ranged from 0.72 to 3.23 for cox-2 and 1.03 to 3.03 for ldlr. There was up-regulation of cox-2 in 6 of 11 tumors compared with normal mucosa. Interestingly, only the tumors with up-regulation of ldlr also had up-regulation of cox-2. Figure 4 shows the ethidium bromide–stained gels for 6 patients with up-regulation of both cox-2 and ldlr compared with the internal standard cyclophilin. This up-regulation did not correlate with the stage or degree of differentiation of the carcinoma in this limited number of specimens (Table II). Total serum cholesterol levels obtained from medical records did not correlate with the degree of up-regulation. However, the 3 patients with stage D carcinoma tended to have lower serum cholesterol (Table II); cholesterol levels were not available for all subjects. All but 1 patient denied taking any aspirin or NSAIDs. Figure 5, which correlates the data by plotting cox-2 against ldlr, shows a linear relationship between cox-2 and ldlr expression. After removal of the 1 outlier (Fig. 5), a significant correlation of 0.74 of the samples was achieved, p = 0.0128.

Two patients who underwent colonoscopic biopsy of neoplasms were found to have only tubular adenomas after the histopathology was confirmed. Relative pixels as densitometric values for the ratio of adenoma mRNA to the paired normal mucosa mRNA for cox-2 and ldlr were 2.90:2.07 for polyp 1 and 2.10:1.67 for polyp 2. It appeared that both cox-2 and ldlr were up-regulated compared with normal colonic tissue in both of the adenomatous polyps. These data were not included in Figure 5.

FIGURE 3 – DiFi growth regulated by LDL. DiFi cells were grown for 48 hr in (1) 1% FCS, (2) 1% FCS + LDL antibody, (3) 10% LPDS medium, (4) 10% LPDS medium + LDL or (5) 10% LPDS medium + LDL + LDLr antibody. Growth was measured by cell counting with a Coulter (Hialeah, FL) counter after trypsinization.

DISCUSSION

We report up-regulation of cox-2 in 6 of 11 colorectal cancer patients. To expand the mechanism of cox-2 up-regulation in colorectal carcinomas, we measured ldlr mRNA expression in these same patients. Caruso et al. (1993) demonstrated the presence of ldlr in colorectal carcinoma by immunoenzymatic assay in about 25% of patients studied. Guddari et al. (1993) showed up-regulation of ldlr mRNA in the human lung adenocarcinoma
TABLE II - PAIRED SPORADIC ADENOCARCINOMA AND NORMAL MUCOSA FROM SURGICAL RESECTIONS OR COLONOSCOPY

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Gender (M/F)</th>
<th>NSAIDs</th>
<th>Cholesterol</th>
<th>Site</th>
<th>Stage</th>
<th>Histology</th>
<th>cox-2</th>
<th>ldlr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>M</td>
<td>No</td>
<td>192</td>
<td>Cecum</td>
<td>B</td>
<td>Moderate</td>
<td>0.72</td>
<td>1.16</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>M</td>
<td>No</td>
<td>158</td>
<td>Cecum</td>
<td>D</td>
<td>Moderate–well</td>
<td>1.61</td>
<td>1.51</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
<td>M</td>
<td>Yes</td>
<td>289</td>
<td>Cecum</td>
<td>A</td>
<td>Well</td>
<td>0.90</td>
<td>1.18</td>
</tr>
<tr>
<td>4</td>
<td>67</td>
<td>M</td>
<td>No</td>
<td>148</td>
<td>Sigmoid</td>
<td>D</td>
<td>Moderate</td>
<td>1.50</td>
<td>3.03</td>
</tr>
<tr>
<td>5</td>
<td>76</td>
<td>M</td>
<td>No</td>
<td>—</td>
<td>Sigmoid</td>
<td>A</td>
<td>Moderate</td>
<td>3.23</td>
<td>1.52</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>M</td>
<td>No</td>
<td>—</td>
<td>Rectum</td>
<td>B</td>
<td>Moderate–well</td>
<td>1.30</td>
<td>1.30</td>
</tr>
<tr>
<td>7</td>
<td>61</td>
<td>M</td>
<td>No</td>
<td>—</td>
<td>Sigmoid</td>
<td>D</td>
<td>Moderate–well</td>
<td>2.92</td>
<td>1.60</td>
</tr>
<tr>
<td>8</td>
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<td>M</td>
<td>No</td>
<td>—</td>
<td>Rectum</td>
<td>B</td>
<td>Well</td>
<td>0.89</td>
<td>1.03</td>
</tr>
<tr>
<td>9</td>
<td>86</td>
<td>M</td>
<td>No</td>
<td>—</td>
<td>Rectum</td>
<td>C</td>
<td>Moderate–well</td>
<td>1.51</td>
<td>1.65</td>
</tr>
<tr>
<td>10</td>
<td>68</td>
<td>M</td>
<td>No</td>
<td>—</td>
<td>Rectum</td>
<td>C</td>
<td>Poor</td>
<td>0.96</td>
<td>1.32</td>
</tr>
<tr>
<td>11</td>
<td>76</td>
<td>M</td>
<td>No</td>
<td>—</td>
<td>Sigmoid</td>
<td>C</td>
<td>Moderate</td>
<td>3.00</td>
<td>1.72</td>
</tr>
</tbody>
</table>

Values for cox-2 and ldlr represent relative pixel densitometry–determined expression of cox-2 mRNA and ldlr mRNA in carcinoma compared with paired normal mucosa. All values are controlled to the relative pixel densitometry–determined expression of the internal standard for β-actin or cyclophilin.

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**Figure 4** - Ethidium bromide staining of RT-PCR products from patient biopsies. Six patients (2, 4, 6, 8, 10, 12) show up-regulation of both cox-2 and ldlr mRNA from tumor specimens compared with paired normal colonic mucosa specimens from the same patient (1, 3, 5, 7, 9, 11). Cyclophilin (CHPI) was used as an internal standard.

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**Figure 5** - Correlation of cox-2 with ldlr message. Percent of control cox-2 mRNA expression is plotted against percent of control ldlr expression. Data are normalized to cyclophilin mRNA level. After removal of the outlier, the correlation coefficient is 0.75 (95% CI); r is significant at p = 0.0128.

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cell line A549. We now demonstrate that LDLr in a colorectal tumor has lost feedback regulation. Several classes of lipoproteins, including LDL, very-low-density lipoproteins and chylomicron remnants, are taken up by the LDLr. Habenicht et al. (1990) demonstrated in fibroblasts that LDLr delivers the essential fatty acid arachidonic acid for PGE2 production. They also showed, in normal cells, that an LDLr-dependent feedback mechanism inhibited the activity of prostaglandin G/H synthase-2 (PGHS; i.e., COX-2), which is a key enzyme in the biosynthetic pathway leading to formation of prostaglandins. In addition to the maintenance of cellular cholesterol homeostasis, the data of Habenicht et al. (1990) indicated that the LDLr pathway has a regulatory role in prostaglandin synthesis. Expression of PGHS by immunoblot assay in colorectal carcinomas has been demonstrated by Kargman et al. (1995).

There are conflicting data on the relationship between total serum cholesterol level and incidence of colorectal carcinoma. Several studies have found that a low serum cholesterol level was associated with increased deaths from colon carcinoma (Williams et al., 1981). Other studies have failed to confirm this inverse relationship (Miller et al., 1981). The mechanism by which serum lipoproteins might potentiate the development of colorectal carcinoma has been hypothesized to be related to COX-2.

Clinical and epidemiological observations regarding the inverse relationship between the use of NSAIDs and a lower incidence of colorectal carcinoma have received attention (Thun et al. 1991; Giovannucci et al., 1994). Most of these studies have demonstrated a reduction in the incidence of colorectal carcinoma in cohorts of patients taking aspirin. Various studies have also confirmed the effect of NSAIDs in polyposis regression in patients with FAP (Giardiello, 1993; Labayle et al., 1991). The exact mechanisms by which NSAIDs cause the regression of polyps are unknown. However, COX enzyme inhibition leading to a reduction of prostaglandins and thromboxanes has been studied. COX is the key regulatory enzyme in the arachidonic acid pathway that is blocked by NSAIDs. There are 2 COX enzymes in humans: COX-1 and COX-2. COX-2 is a mitogen-inducible enzyme, shown by Eberhart et al. (1994) to be up-regulated in over 75% of human adenocarcinoma patients and in just under 50% of colorectal adenomas. The mechanism of action of COX-2 up-regulation in these tumors is unknown; however, we have reported that newly synthesized PGE2 up-regulates cox-2 in colorectal and prostate tumor lines (Tjandrawinata and Hughes-Fulford, 1997; Tjandrawinata et al., 1997). Based on our findings that COX-2 is a feed-forward enzyme, we hypothesized that if the LDLr was not normally regulated, these tumors would import more essential fatty acids and there would be a correlation of ldlr and cox-2 message expression. We first tested our hypothesis that the LDLr may not be regulated in tumor cells using the DiFi cell line, where we found that the tumor cells had lost normal LDLr regulation as tested by measuring LDL uptake using a 96 well fluorometer and microscopic studies. We found no feedback regulation in the tumor cell line (data not shown). We observed the same abnormal regulation of the ldlr in prostate tumor
Addition of LDL to DiFi cells grown in LPDS did not affect expression of the functional LDLr protein or mRNA even after 24 hr. In addition, LDL was necessary for normal growth. When LDLr blocking antibody was added to growing cells, growth was significantly inhibited. We do not yet know the cause of the lack of feedback regulation of LDLr. A lack of feedback regulation has been noted in Burkitt’s lymphoma cells (Yen et al., 1995), while Axelson and Larsson (1996) reported altered metabolism of LDL cholesterol in malignant cells, including breast carcinoma, colonic carcinoma and malignant melanoma.

In addition to the finding that LDLr is abnormally up-regulated, there is coordinate up-regulation of Cox-2 mRNA in a significant number of colorectal adenocarcinomas, with no up-regulation of either gene in normal mucosa of the same patients. These studies suggest that the up-regulation of ldlr mRNA in certain human colorectal adenocarcinomas could lead to inappropriate cellular uptake of essential fatty acids, such as linoleic acid or arachidonic acid, resulting in high levels of cellular eicosanoids. This in turn could lead to up-regulation of COX-2 (a known feed-forward enzyme in tumors) (TjandraWINATA and Hughes-F Fulford 1997; TjandraWINATA et al., 1997) with a subsequent increase in prostaglandin synthesis. Up-regulation of Cox-2 by both PGE2 precursors, linoleic acid and arachidonic acid, has also been seen in DiFi cells (data not shown). Thus, high-fat diets may promote growth in tumors with up-regulation of LDLr. Our hypothesis does not preclude other proposed mechanisms, such as high-fat-induced alteration of tumor-promoting searcy bile acid (deoxycholic acid and lithocholic acid) content of the colon and its subsequent increase in colonic cellular proliferation and other tumor-promoting properties.

Efforts continue to achieve a better understanding of the relationship of LDLr and Cox-2 expression in colorectal carcinoma, adenomas and human cancer cell lines. Since LDLr is the primary path of arachidonic acid uptake, further studies of its relationship with growth regulation of cancer cells, with PGE2 synthesis through COX-2 as well as with high-fat diets may eventually determine whether the LDLr is a potential target site for chemoprevention of certain colorectal carcinomas.

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REFERENCES


