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

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**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 *ldlr* mRNA lacks normal feedback regulation in some colorectal cancers. Analysis of the *ldlr* 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 *ldlr* and its co-regulation with *cox-2* in normal and tumor specimens from patients with colorectal adenocarcinomas. The ratio of tumor:paired normal mucosa of mRNA expression of *ldlr* and of *cox-2* was measured in specimens taken during colonoscopy. *ldlr* and *cox-2* transcripts were apparent in 11 of 11 carcinomas. There was significant coordinate up-regulation both of *ldlr* 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 *ldlr*. 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.*
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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, lipoxygenase and cyclo-oxygenase 1 (COX-1) or COX-2. The COX pathway leads to production of various prostaglandins, including $PGE_2$. LDLrs play a key regulatory role in the initial uptake of essential fatty acid into cells and the subsequent synthesis of eicosanoids such as $PGE_2$ (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; Tjandrawinata and Hughes-Fulford, 1997; Tjandrawinata *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 *ldlr* 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 *ldlr* 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 *ldlr* 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 LDLrs. The current study suggests that *ldlr* 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 DiI-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

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% $CO_2$ 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% $CO_2$ and fed 3 times a week.

## Measurement of DiI 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 $1.5 \times 10^5$ cells/well and grown in 2% LPDS-containing medium for 24 hr; 6 µg 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, DiI-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 DiI-LDL:LDL molar concentration equivalent. Cells were rinsed 4 times with PBS, and DiI-LDL uptake was quantified with a Fluoroskan II fluorometer (Labsystems; Needham Heights, MA) based on the method described by Stephan and Yurachek (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 DiI-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 of each 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 portion was carried out in a total volume of 50 µl containing 5 µl of single-stranded cDNA from RT sample, 2.5 mM $MgCl_2$ (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′, CAATGTCTCACCAAGCTCT, anti-sense, 5′ to 3′, TCTGTCTCGAGGGGTAGCT. Primers used for cox-2 were as follows: sense, 5′ to 3′, CCCCATAACCCCGC-CAAAAGG, anti-sense, 5′ to 3′, AAAATAGGAGAAAC-GAAGTGT. Primers used for the internal standard cyclophilin were as follows: sense, 5′ to 3′, CGTCTCCTTTGAGCTGTTTG-CAGAC, anti-sense, 5′ to 3′, CATAATCATAAACTTAACTCTG-CAATCCAGC. 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. RT 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 ethidium bromide, and photographed with a Polaroid camera over UV light. For quantification, the bands of interest were scanned at 400 dpi (Scanjet Iicx; Hewlett-Packard, Palo Alto, CA) and stored as Macintosh TIFF files. Peak areas and densities were determined using NIH Image 1.55 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 DiI-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).

### LDL 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 LDL, 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,
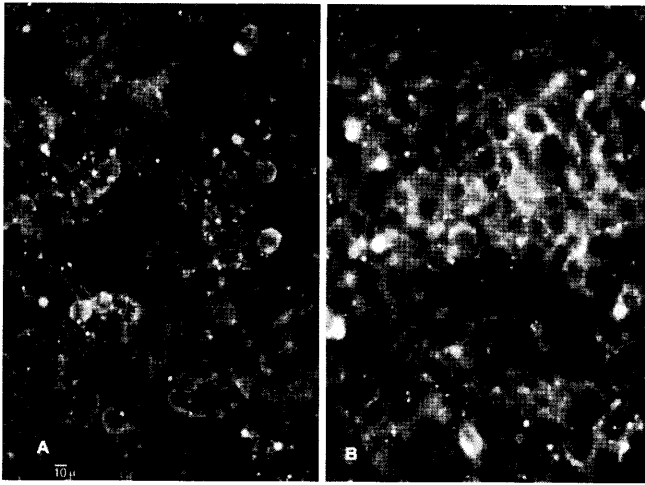
**FIGURE 1** – LDLr-mediated uptake of DiI-labeled LDL in DiFi cells. LDL uptake is seen in the bright areas. Normal regulation of the LDLr would be seen by a reduced uptake of LDL. Cells were grown for 24 hr in 2% LPDS before medium was supplemented with LDL. Cells were then grown in 2% LPDS *(a)* without LDL or *(b)* with LDL (6 µg/ml) for 24 hr before addition of DiI-LDL. Cold LDL (6 µg/ml) was added to the samples lacking LDL during the 24-hr incubation with the DiI-LDL fluorescent label (90 min) to keep the DiI-LDL:LDL molar concentration equivalent.

**TABLE I** – LDL UPTAKE IN DiFi CELLS[1]

| Experimental condition | Relative fluorescence per well |
|---|---|
| −LDL | $5.9 \pm 1.2$ |
| +LDL | $6.8 \pm 2.3$ |

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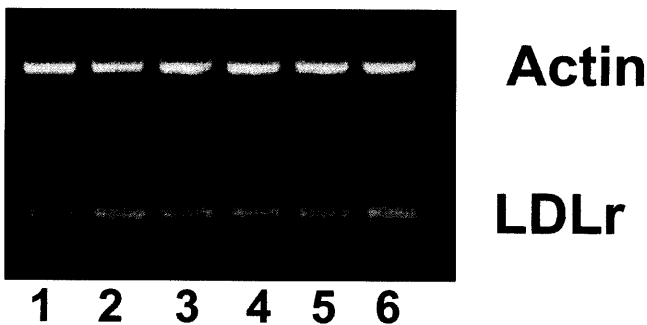


**FIGURE 2** – *ldlr* mRNA is not regulated by LDL in DiFi cells. Representative expression of the *ldlr* message in DiFi cells visualized on an ethidium bromide gel. Cells were grown as described above, then incubated with LDL for (1) 0 hr, (2) 1 hr, (3) 2 hr, (4) 6 hr, (5) 12 hr or (6) 24 hr. mRNA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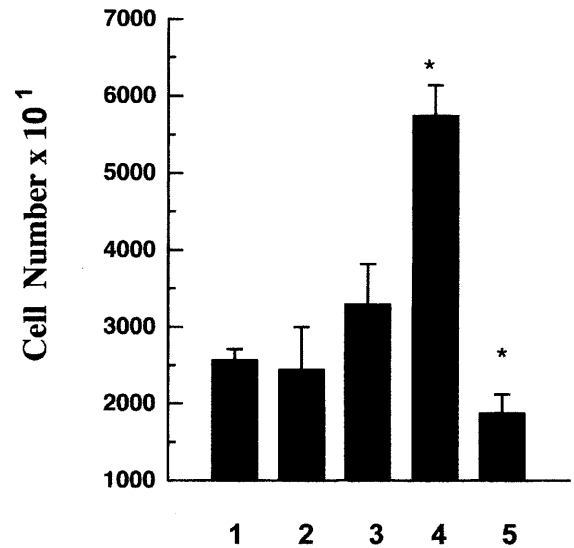


**FIGURE 3** – DiFi growth regulated by LDL. DiFi cells were grown for 48 hr in (1) 1% FCS, (2) 1% FCS + LDL antibody, (3) 10% LPDS medium, (4) 10% LPDS medium + LDL or (5) 10% LPDS medium + LDL + LDLr antibody. Growth was measured by cell counting with a Coulter (Hialeah, FL) counter after trypsinization.

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.

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 immuno-enzymatic assay in about 25% of patients studied. Gueddari *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

| Subject | Age (years) | Gender (M/F) | NSAIDs | Cholesterol | Site | Stage | Histology | cox-2 | ldlr |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 80 | M | No | 192 | Cecum | B | Moderate | 0.72 | 1.16 |
| 2 | 73 | M | No | 158 | Cecum | D | Moderate–well | 1.61 | 1.51 |
| 3 | 77 | M | Yes | 289 | Cecum | A | Well | 0.90 | 1.18 |
| 4 | 67 | M | No | 148 | Sigmoid | D | Moderate | 1.50 | 3.03 |
| 5 | 36 | M | No | — | Sigmoid | A | Moderate | 3.23 | 1.52 |
| 6 | 70 | M | No | — | Rectum | B | Moderate–well | 1.30 | 1.30 |
| 7 | 61 | M | No | 157 | Sigmoid | D | Moderate–well | 2.92 | 1.60 |
| 8 | 69 | M | No | — | Rectum | B | Well | 0.89 | 1.03 |
| 9 | 86 | M | No | — | Rectum | C | Moderate–well | 1.51 | 1.65 |
| 10 | 68 | M | No | — | Rectum | C | Poor | 0.96 | 1.32 |
| 11 | 76 | M | No | — | Sigmoid | C | Moderate | 3.00 | 1.72 |

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.
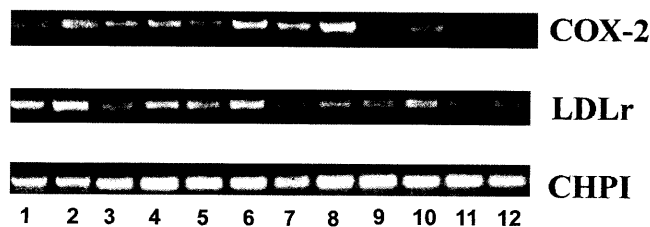


FIGURE 4 – Ethidium bromide staining of RT-PCR products from patient biopsies. Six patients (2, 4, 6, 8, 10, 12) show up-regulation of both cox-2 and ldlr mRNA from tumor specimens compared with paired normal colonic mucosa specimens from the same patient (1, 3, 5, 7, 9, 11). Cyclophilin (CHPI) was used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

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 $PGE_2$ 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 polyp 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
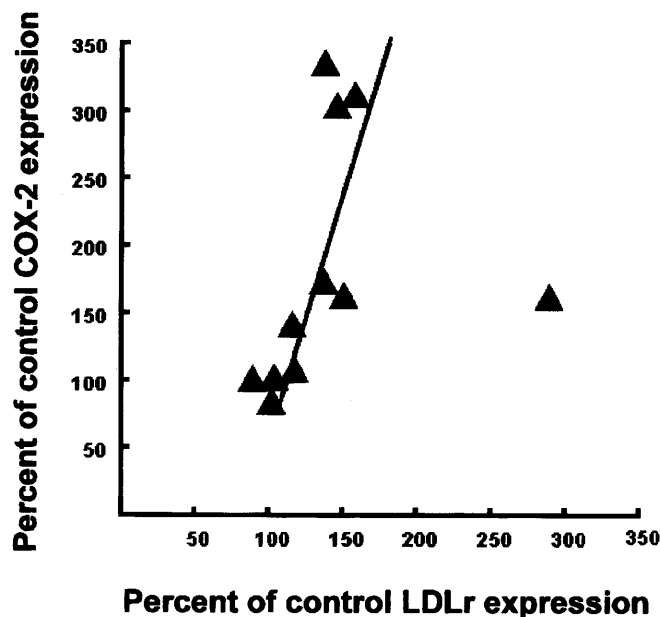


**Percent of control LDLr expression**

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 $p = 0.0128$.

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 $PGE_2$ 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

cells. 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 LDL. 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-Fulford 1997; Tjandrawinata *et al.,* 1997) with a subsequent increase in prostaglandin synthesis. Up-regulation of *cox-2* by both $PGE_2$ 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 secary 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 arachidomic acid uptake, further studies of its relationship with growth regulation of cancer cells, with $PGE_2$ 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.

REFERENCES

AWAD, A.B., FINK, C.S. and HORVATH, P.J., Alteration of membrane fatty acid composition and inositol phosphate metabolism in HT-29 human colon cancer cells. *Nutr. Cancer,* **19,** 181–190 (1993).

AXELSON, M. and LARSSON, O., 27-Hydroxylated low density lipoprotein (LDL) cholesterol can be converted to 7alpha,27-dihydroxy-4-cholesten-3-one (cytosterone) before suppressing cholesterol production in normal human fibroblasts. Evidence that an altered metabolism of ldl cholesterol can underline a defective feedback control in malignant cells. *J. biol. Chem.,* **31,** 12724–12736 (1996).

CARUSSO, M.G., NOTARNICOLA, M., CAVALLINI, A., GUERRA, V., MISCIANGNA, G. and DI LEO, A., Demonstration of low density lipoprotein receptor in human colonic carcinoma and surrounding mucosa by immuno-enzymatic assay. *Ital. J Gastroenterol.,* **25,** 361–367 (1993).

EBERHART, C.E., COFFEY, R.J., RADHIKA, A., GIARDIELLO, F.M., FERRENBACH, S. and DUBOIS, S.R., Up-regulation of cyclo-oxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology,* **107,** 1183–1188 (1994).

GIARDIELLO, F.M., HAMILTON, S.R., KRUSH, A.J., PIANTADOSSI, S., HYLIND, L.M., CELANO, P., BOOKER, S.V., ROBINSON, C.R. and OFFERHAUS, G.J.A., Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. *N. Engl. J. Med.,* **328,** 1313–1316 (1993).

GIOVANNUCCI, E., RIMM, E.B., STAMPFER, M.J., COLDITZ, G.A., ASCHERIO, A. and WILLIET, W.C., Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. *Ann. intern. Med.,* **121,** 241–246 (1994).

GROSS, M.E., ZORBAS, M.A., DANIELS, Y.J., GARCIA, R., GALLICK, G.E., BRATTAIN, M.G., BOMAN, B.M. and YEOMAN, L.C., Cellular growth response to epidermal growth factor in colon carcinoma cells with an amplified epidermal growth factor receptor derived from a familial adenomatous polyposis patient. *Cancer Res.,* **51,** 1452–1459 (1991).

GUEDDARI, N., FAVRE, G., HACHEM, H., MAREK, E., LE GAILLARD, F. and SOULA, G., Evidence for up-regulated low density lipoprotein receptor in human lung adenocarcinoma cell line A549. *Biochimie,* **75,** 811–819 (1993).

HABENICHT, A.J.R., SALBACH, P., GOERIG, M., ZEH, W., JANSSEN-TIMMEN, U., BLATTNER, C., KING, W.C. and GLOMSET, J.A., The LDL receptor pathway delivers arachidonic acid for eicosanoid formation in cells stimulated by platelet-derived growth factor. *Nature (Lond.),* **345,** 634–636 (1990).

HABENICHT, A.J.R., SALBACH, P. and JANSSSEN-TIMMEN, U., LDL receptor–

dependent polyunsaturated fatty acid transport and metabolism. *Eicosanoids,* **5 (Suppl.),** 529–531 (1992).

KARGMAN, S.L., O'NEILL, G.P., VICKERS, P.J., EVANS, J.F., MANCINI, J.A. and JOTHY, S., Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. *Cancer Res.,* **55,** 2556–2559 (1995).

LABAYLE, D., FISCHER, D., VIELH, P., DROUHIN, F., PARIENTE, A., BORIES, C., DUHAMEL, O., TROUSSET, M. and ATTALI, P., Sulindac causes regression of rectal polyps in familial adenomatous polyposis. *Gastroenterology,* **101,** 635–639 (1991).

MILLER, S.R., TARTTER, P.I., PAPATESTAS, A.E., SLATER, G. and AUFSES, A.H., JR., Serum cholesterol and human colon cancer. *J. nat. Cancer Inst.,* **67,** 297–300 (1981).

NICHOLSON, M.L., NEOPTOLEMOS, J.P., CLAYTON, H.A., TALBOT, I.C. and BELL, P.R.F., Increased cell membrane arachidonic acid in experimental colorectal tumors. *Gut,* **32,** 413–418 (1991).

REDDY, B.S., Dietary fat and colon cancer: animal model studies. *Lipids,* **27,** 807–813 (1992).

STEPHAN, Z.F. and YURACHEK, E.C., Rapid fluorometric assay of LDL receptor activity by DiI-labeled LDL. *J. Lipid Res.,* **34,** 325–330 (1993)

THUN, M.J., NAMBOODIRI, M.M. and HEATH, C.W., JR., Aspirin use and reduced risk of fatal colon cancer. *N. Engl. J. Med.,* **325,** 1593–1596 (1991).

TJANDRAWINATA, R. and HUGHES-FULFORD, M., Upregulation of cyclo-oxygenase 2 by-product, prostaglandin $E_2$ in prostate cancer. *Adv. exp. Med. Biol.,* **407,** 163–170 (1997).

TJANDRAWINATA, R., RAJVIR, D. and HUGHES-FULFORD, M., Induction of cyclo-oxygenase 2 mRNA by prostaglandin $E_2$ in prostate cancer. *Brit. J. Cancer,* **75,** 1111–1118 (1997).

WILLIAMS, R.R., SORLIE, P.D., FEINLEIB, M., MCNAMURA, P.M., KANNEL, W.B. and DAWBER, T.R., Cancer incidence by levels of cholesterol. *J. Amer. med. Ass.,* **245,** 247–252 (1981).

WINGO, P.A., TONG, T. and BOLDEN, S., Cancer statistics. *CA Cancer J. Clin.,* **8,** 8–30 (1995).

YEN, C.F., KALUNTA, C.I., CHEN, F.S., KAPTEIN, J.S., LIN, C.K. and LAD, P.M., Regulation of low-density lipoprotein receptors and assessment of their functional role in Burkitt's lymphoma cells. *Biochim. biophys. Acta,* **1257,** 47–57 (1995).

YEUNG, K.S. and 14 OTHERS, Comparison of diet and biochemical characteristics of stool and urine between Chinese populations with low and high colorectal cancer rates. *J. nat. Cancer Inst.,* **83,** 46–50 (1991).