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Fatty acid regulates gene expression and growth of human prostate cancer PC-3 cells

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It has been proposed that the ω-6 fatty acids increase the rate of tumor growth. Here we test that hypothesis in the PC-3 human prostate tumor. We found that the essential fatty acids, linoleic acid (LA) and arachidonic acid (AA), and the AA metabolite PGE_2 stimulate tumor growth while oleic acid (OA) and the ω-3 fatty acid, eicosapentaenoic acid (EPA) inhibited growth. In examining the role of AA in growth response, we extended our studies to analyze changes in early gene expression induced by AA. We demonstrate that c-*fos* expression is increased within minutes of addition in a dose-dependent manner. Moreover, the immediate early gene *cox-2* is also increased in the presence of AA in a dose-dependent manner, while the constitutive *cox-1* message was not increased. Three hours after exposure to AA, the synthesis of PGE_2 via COX-2 was also increased. Previous studies have demonstrated that AA was primarily delivered by low density lipoprotein (LDL) via its receptor (LDLr). Since it is known that hepatomas, acute myelogenous leukemia and colorectal tumors lack normal cholesterol feedback, we examined the role of the LDLr in growth regulation of the PC-3 prostate cancer cells. Analysis of *ldlr* mRNA expression and LDLr function demonstrated that human PC-3 prostate cancer cells lack normal feedback regulation. While exogenous LDL caused a significant stimulation of cell growth and PGE_2 synthesis, no change was seen in regulation of the LDLr by LDL. Taken together, these data show that normal cholesterol feedback of *ldlr* message and protein is lost in prostate cancer. These data suggest that unregulated over-expression of LDLr in tumor cells would permit increased availability of AA, which induces immediate early genes c-*fos* and *cox-2* within minutes of uptake.

Introduction

Essential fatty acids (EFA) and their products (including prostaglandins) have been implicated in upregulation of tumor cell growth (1,2). Prostaglandins (PGs) are short-lived and act in an autocrine or paracrine manner to convey their biological effects during cell growth, differentiation or inflammation. Transformation of arachidonic acid (AA) into prostaglandins (PG) and tromboxanes (TX) is catalyzed by the enzyme cyclooxygenase (COX; EC 1.14.99.1). Previous studies have demonstrated that there are two forms of cyclooxygenase, *cox-1*, which is constitutive, and *cox-2*, which is an immediate early gene and is inducible. Cox-2 is upregulated in prostate cancer biopsies (3,4). Cellular levels of free AA, in turn, are controlled by a series of reactions by which the fatty acid (FA) is released from membrane phospholipids by phospholipase A_2 (PLA_2) and esterified in the phospholipids by acyltransferases. Since cellular AA is almost exclusively sequestered in phospholipids, the availability of this fatty acid is important for the synthesis of PGs. There are two primary means by which cells can acquire AA: (i) from AA–albumin complex that is distributed freely in serum (5), and (ii) from serum lipoprotein, which sequesters unsaturated fatty acids, and is taken up by the cell through a low density lipoprotein receptor (LDLr)-mediated mechanism (6). Habenicht and co-workers proposed that AA originates from LDL cholesteryl esters and can be metabolized by a variety of enzymes including COX and lipoxygenase (LOX) into PGs, TXs, and leukotrienes (LTs), respectively (6–9).

Epidemiological, animal and cell studies have linked the essential fatty acids with prostate tumor growth (10–13). There is evidence of a positive correlation between the levels of LDL and cancer cell growth. Studies by Bayerdorffer *et al.* (14) demonstrated that a higher LDL and VLDL cholesterol level is associated with the development of colorectal adenomas, the benign precursors of colorectal cancer. The level of expression of LDLr mRNA is found to be upregulated in lung adenocarcinoma cells (15), colorectal carcinoma (16,17) and leukemia cells (18,19). We have recently demonstrated that the up-regulation of *cox-2* in colorectal tumors is co-regulated with LDLr expression in human tumor biopsies and human tumor cells (16). Low sera cholesterol has long been associated with increased cancer morbidity (20). To say that low cholesterol is a risk factor for cancer is in conflict with data from Japanese studies where subjects had lower plasma cholesterol level and a low incidence of many cancers. Moreover, patients on drugs that lower endogenous cholesterol synthesis show no increased incidence of cancer, despite the lowering of sera cholesterol (21). This dichotomy may be explained by studies which demonstrated that lower cholesterol is not a risk factor, but rather a consequence of the tumor. In a prospective study of 25 patients with colorectal carcinoma, individual shifts of sera cholesterol after surgery were measured. There was a significant rise in plasma cholesterol levels in patients after curative surgery, but not after non-curative surgery. Since an elevated level of LDL receptor was found in the tumor when compared with the tumor-free margin, it was postulated that low cholesterol levels in cancer patients were a consequence of, not the cause of malignancy (17).

This laboratory has previously reported that PGE_2 plays a role in the up-regulation of cell growth and *cox-2* expression

Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; $dmPGE_2$, 16,16-dimethyl PGE_2; EFA, essential fatty acids; FA, fatty acid; FCS, fetal calf serum; LDL, low density lipoprotein; LDLr, low density lipoprotein receptor; LOX, lipoxygenase; LPDS, lipoprotein-deficient serum; LTs, leukotrienes; PGs, prostaglandins; PLA_2, phospholipase A_2; PPARs, peroxisome proliferator-activated receptors; PPRE, peroxisome proliferator receptor element; PUFA, polyunsaturated fatty acids; TXs, thromboxanes.

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in the human prostate cancer cell line PC-3 (22). A second study from this laboratory shows that AA mediates induction of immediate early gene c-*fos* via new endogenous synthesis of PGE_2 and stimulation of the prostaglandin EP-4 receptor (23). The current study demonstrates that essential fatty acids and their product PGE_2 stimulate growth of human prostate carcinoma cells. It also suggests that a loss of LDLr feedback regulation in tumors allows an avenue of FA delivery to the cell which in turn upregulates growth related genes and tumor growth, linking specific fatty acids with regulation of tumor growth.

Materials and methods

LDL isolated from human plasma was purchased from Sigma Chemical (St Louis, MO). Fluorescent DiI–LDL probe was purchased from Molecular Probes (Eugene, OR). 16,16-Dimethyl PGE_2 ($dmPGE_2$) was from Cayman Chemical (Ann Arbor, MI). RPMI-1640 medium, L-glutamine and trypsin were from UCSF Cell Culture Facility (San Francisco, CA). Fetal calf serum (FCS) was from Gibco BRL (Gaithersburg, MD). Human lipoprotein-deficient serum (LPDS), fatty acid free albumin (FFA), 99% pure AA, LA, OA, PGE_2 and EPA as well as antibiotic-antimycotic solution (containing penicillin, streptomycin and amphotericin B) were purchased from Sigma Chemical (St Louis, MO).

Cell culture

Normal human fibroblasts GM03348D were obtained from Coriell Cell Repository (Camden, NY) and grown in 10% FCS-containing RPMI-1640 medium supplemented with L-glutamine and antibiotics. Human prostatic carcinoma PC-3 cells obtained from either ATCC or UC cell culture facility were grown in the same media supplemented with glucose (25 mM) and pyruvate (1 mM). Cells were maintained at high density in a 37°C incubator with 5% CO_2 and fed three times a week. Twenty-four hours prior to cell platings; cell stocks were fed with fresh 10% FCS-containing medium. $DmPGE_2$ was used since this PGE_2 analog is a stable compound with the equivalent biological activity. Longer incubation of native PGE_2 results in the breakdown of the compound and therefore the use of a stable analog is necessary for long periods of incubations. A side benefit of using $dmPGE_2$ is that subsequent analysis of newly synthesized PGE_2 can be made on these cells since $dmPGE_2$ is not detected by the assay. Eicosanoids were made fresh as stocks in 10 mg/ml ethanol; in some cases they were stored at –20°C under oxygen-free conditions. Cells were down-regulated for 24 h in 2% FCS MEM media with 1.25 mg/ml albumin with additional 25 mM glucose and 1 mM pyruvate before fatty acids were added directly to the depleted media. Alcohol content did not exceed 1 µg/ml for any experiment. Controls had albumin/ethanol added without eicosanoid. Quantification of cell number was accomplished either by CyQuant or by Coulter counter techniques.

PGE_2 analysis

The exogenous PGE_2 levels were quantitated by using PGE_2-Monoclonal Enzyme Immunoassay kit (Cayman Chemical; Ann Arbor, MI) according to the protocol recommended by the manufacturer. This assay system is very specific for native PGE_2 and does not detect $dmPGE_2$ or any prostaglandin of other series (A,B, D and F). The samples contained in the 96-well plate was read at 410 nm using the Dynatech MR5000 Microplate Reader (Dynatech Laboratories; Chantilly, VA) and the data were analyzed with the BioLinx 2.0 Software (Dynatech Laboratories) run on an IBM-compatible PC.

RNA isolation and RT–PCR

RNA was extracted and purified by the acid guanidium thiocyanate/phenol/chloroform extraction method (RNA Stat-60 reagent) described previously (22). RNA was reverse-transcribed and PCR was carried out in Robocycler 40 Temperature Cycler (Stratagene; San Diego, CA) as described previously (20). The primers used for priming the LDLr gene were, 5′-CAA TGT CTC ACC AAG CTC TG (sense) and 5′-TCT GTC TCG AGG GGT AGC TG (anti-sense); and for internal standard β-actin, 5′-CCG CAA ATG CTT CTA GGC (sense) and 5′-GGT CTC ACG TCA GTG TAC GG (anti-sense). We have described the *cox-2* primers previously (22,23). All primers used were tested for linearity over cycle number; thereafter analyses were carried out in the linear portion of the curve, therefore allowing semi-quantitative analysis of gene induction. PCR bands were identified by size after electrophoresis on a 2% agarose gel in TAE buffer. Ethidium bromide stained bands were photographed with an instant camera DS-34 (Polaroid, Cambridge, MA) under UV light.

Table I. PC-3 cell growth in the absence or presence of LDL or AA

Condition	Cell growth (as percentage of control)	PGE_2 (pg/ml)
Control	100 ± 15	1039
LDL (8 µg/ml)	139 ± 13*	1803
AA (7.5 µg/ml)	222 ± 20**	20 714

PC-3 cells were seeded in 96-well plates with 1% FCS and grown 36 h with and without added LDL or AA. Measurements of growth (by CyQuant™ assay) or PGE_2 were taken at the end of the incubation period (n = 3). Significance: $^*P < 0.05$, $^{**}P < 0.001$ when compared with controls.

Measurement of DiI–LDL uptake

Quantification of DiI–LDL uptake was performed based on the analysis previously described (22,23). Cells were plated in a 96-well plate at a density of 1.5×10^5 cells/well, grown 24 h in 2% FCS before media was changed to 10% LPDS with or without LDL for 24–26 h. After incubation cells were rinsed in albumin containing PBS four times and then Dil-LDL uptake was quantitated with a Fluoroskan II fluorometer (Labsystems, Helsinki, Finland) with equal final molar concentrations of cold LDL added with the DiI–LDL for 30 min. Fluorescence was read with excitation wavelength set at 520 nm (excitation) and the emission at 578 nm (emission). Photographic data were obtained using a Zeiss Axiophot fluorescent microscope with samples used the same DiI and LDL materials from the same lot number on the same day and exposures were made at identical time periods.

Results

LDL and essential fatty acids accelerate growth of PC-3 cells

To investigate the effect of LDL on cell growth and PGE_2 synthesis in prostate cancer cells, PC-3 cells were grown in medium containing LPDS in the presence or absence of exogenous LDL. Table I shows that addition of exogenous LDL (8 µg/ml) increases cell growth 37% and increases cellular PGE_2 almost 2-fold during the 36 h treatment period. Because essential fatty acids carried by LDL are hypothesized to be responsible for increased PGE_2 synthesis and cell growth, we also tested the effect of AA treatment on these two parameters. AA alone stimulated growth almost 2-fold and increased PGE_2 synthesis 20-fold.

It was still possible that the added FA increases growth by increasing available energy sources. Therefore the ability of various fatty acids to stimulate proliferation was tested in the presence of alternative energy sources. Figure 1 shows that after 24 h in low-lipid sera, addition of PGE_2, LA or AA increased growth of human prostate cancer cells. The increase of growth was significant for all three treatments ($P < 0.01$). However, there was no significant difference between the treatments themselves, showing that LA and its metabolites, AA and PGE_2 are equally potent growth stimulators. In contrast, OA and EPA significantly decreased cell numbers ($P < 0.001$ and $P < 0.01$, respectively) suggesting an inhibitory effect by these fatty acids.

Essential fatty acid AA induces immediate early genes

Since AA stimulated cancer cell growth, its effect on induction of early growth genes was examined. AA induced *c-fos* within 30 min of addition (Figure 2). This induction was dose responsive, with maximal stimulation beginning at 5 µg AA/ml. A significant induction ($P < 0.001$) of *cox-2* message occurred 2 h later (Figure 3). The dose response of *cox-2* message induction was paralleled by significant increases in COX-2 protein activity (Figure 4). After incubation with varying amount of AA, the activity of the enzyme was evaluated by measurement of PGE_2 synthesis in presence of

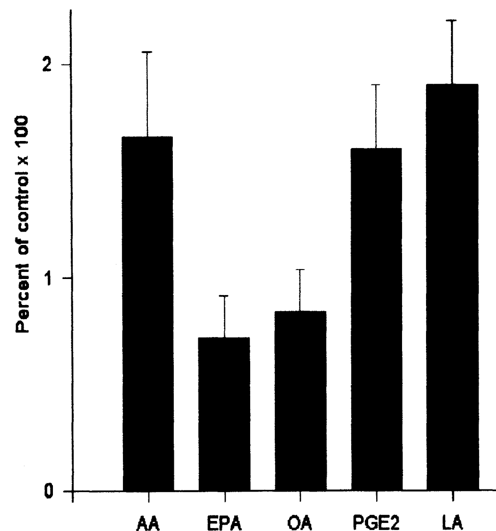

Fig. 1. Regulation of growth by fatty acids: comparison of PC-3 growth induction by FA. PC-3 cells were grown in 2% FCS media for 24 h before addition of FA. After 7.5 µg/ml AA, OA, EPA, PGE_2 or LA were added to depleted media, cells were grown for 24 h before cell counts. Cells were grown in triplicate. Data are presented as percent of control ± SD.

1 µg/ml AA. COX-2 activity (PGE_2 synthesis) was maximal in cells grown with a dose of 1–10 µg AA/ml.

Changes of LDLr mRNA levels in response to LDL and other exogenous factors

Reports have demonstrated a loss of cholesterol feedback in hepatomas (24) with later studies showing a lack of LDL receptor feedback regulation in colorectal cancer (16), acute myelogenous leukemia (18) and Burkitt's lymphoma (19). We suspected that prostate cancer cells also lacked a feedback regulation of LDLr by LDL, which could lead to increased uptake of LDL and subsequent cell growth. The effect of exogenous LDL on LDLr mRNA expression was examined by RT–PCR. Densitometry readings of the resulting PCR products were standardized to the internal control β-actin. Addition of exogenous LDL did not change the levels of LDLr mRNA expression. A representative result of three experiments is shown in Figure 5A. We also used normal human fibroblasts in the same experiment as control. As expected, LDL had feedback regulation on LDLr mRNA expression in normal cells (Figure 5B).

Since FCS and PGE_2 upregulate *cox-2* expression, we asked if they also regulate LDLr expression. Varying FCS concentrations (2 versus 10%) were added to PC-3 cells and expression analyzed using RT–PCR (Figure 6). There was no significant difference in the levels of LDLr mRNA from cells grown in either 2% FCS- (lane 1) or 10% FCS-containing medium (lane 4), indicating that higher concentrations of serum lipoprotein did not downregulate LDLr mRNA expression. Long-term (18 h) and short-term (3 h) effect of administration of exogenous $dmPGE_2$ on LDLr expression was also investigated. As shown in Figure 6, no significant changes in LDLr mRNA levels were seen with either long- or short-term $dmPGE_2$ administration. Taken together, these data suggest that LDLr mRNA expression in PC-3 is not feedback regulated by LDL or by the growth stimulator and FA metabolite, PGE_2.

LDL uptake by PC-3 cells is not regulated by LDL

Fluorescent-labeled DiI–LDL was used as a measurement of LDL uptake by LDLr protein as described previously (22,23).

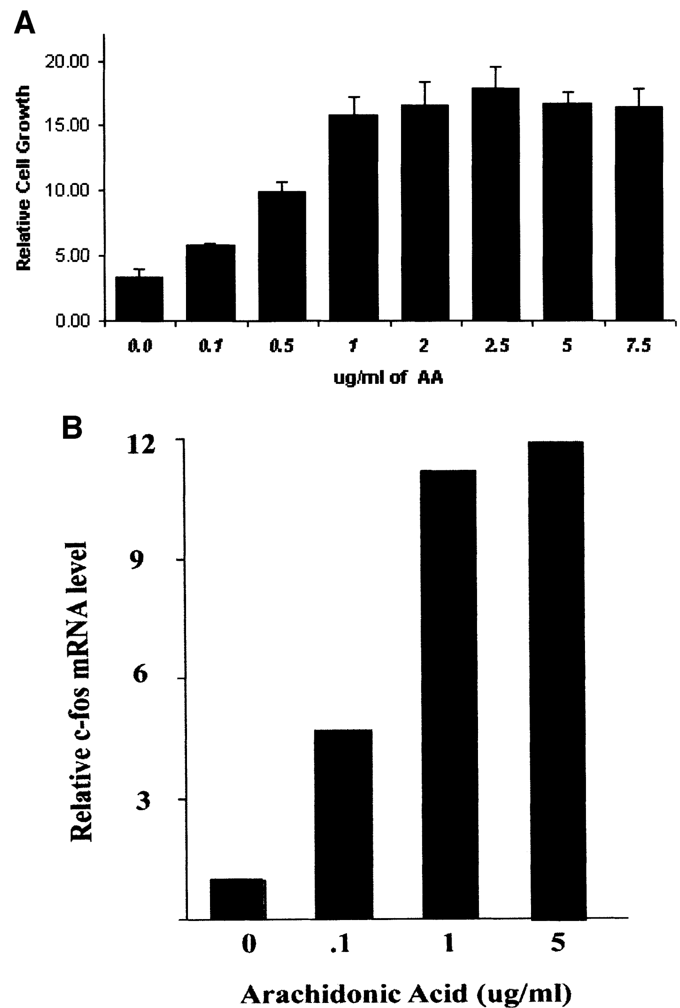

Fig. 2. Arachidonic acid upregulation of growth and *c-fos* gene expression. Cells were grown in 2% FCS media for 24 h before addition of AA. (**A**) AA was added at varying concentrations (0.1, 0.5 1, 2, 2.5, 5 or 7.5 µg/ml), growth was measured using CyQuant™; (**B**) 0.1, 1 or 5 µg/ml AA. RNA was collected at 30 min after addition of treatment and relative *c-fos* expression analyzed by RT–PCR as described in Materials and methods section. The figure is a representative of three separate experiments.

As seen in Table II, the amount of DiI–LDL uptake by cells as measured by spectrofluorometry did not vary significantly in cells grown in LPDS in the absence or presence of exogenously administered LDL. This result was confirmed by examining the localization of uptake of fluorescent DiI–LDL in the cells under the fluorescent microscope. As seen in the photographs in Figure 7, visualization of DiI–LDL uptake is comparable in both treatment groups, i.e. in medium with LPDS in the presence or absence of LDL for 36 h. These results indicate that the LDLr protein is functional, and that it is not feedback regulated by exogenous lipoprotein in PC-3 cells. In contrast, addition of exogenous LDL down-regulated LDL uptake in normal human fibroblasts grown in LPDS.

Discussion

Our long-term interest in finding a biochemical link between cellular fatty acids and cancer was renewed by the observation that exogenous LDL is able to deliver AA for prostaglandin and leukotriene synthesis (9). Previous reports on PGE_2 upregulation of prostate cancer growth and *cox-2* mRNA synthesis

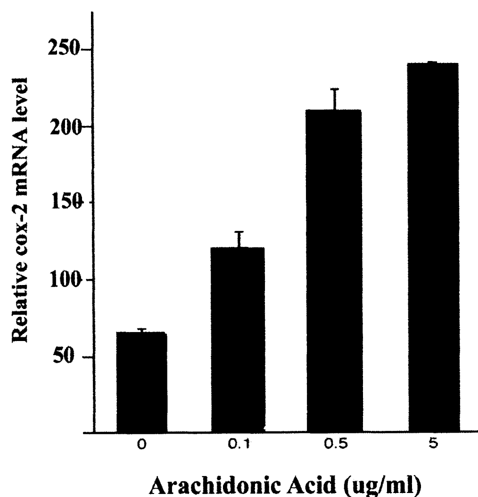

Fig. 3. Arachidonic acid upregulation of *cox-2* mRNA. Cells were grown in 2% FCS media for 24 h before addition of AA. AA was added at varying concentrations (0.1, 0.5, or 5 μg/ml). RNA was collected 2 h after addition of treatment and analyzed by RT–PCR as described in Materials and methods section. The figure is a representative of three separate experiments.

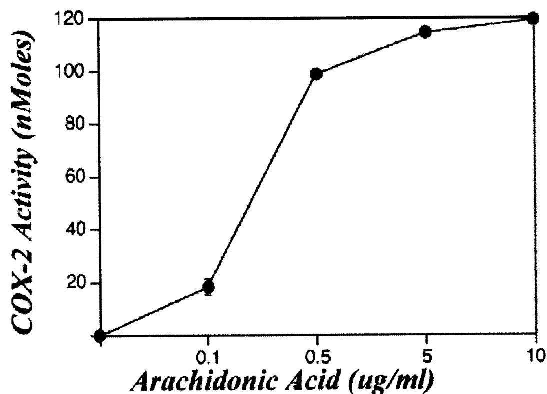

Fig. 4. Arachidonic acid upregulation of COX-2 enzyme activity. Cells were grown in 2% FCS media for 24 h before addition of AA. AA was added at varying concentrations (0.1, 0.5, 5 or 10 μg/ml) for 2.5 h. Media with 1% FCS and 1 μM AA was added to the cells for 30 min, collected and analyzed for synthesis of PGE_2 the COX-2 product. The figure is a representative of three experiments.

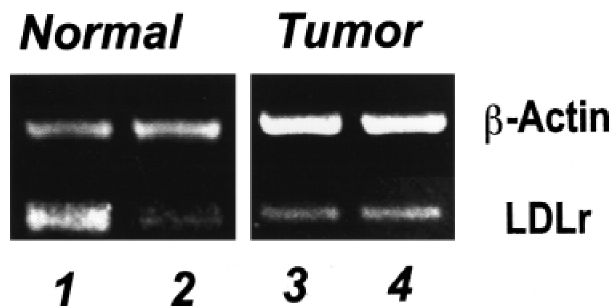

Fig. 5. Comparison of *ldlr* gene expression regulated by LDL. PC-3 cells or normal human fibroblasts were grown in 10% LPDS ± LDL for 24 h of treatment. RNA was isolated and subject to RT–PCR using LDLr or β-actin primers (as internal control). Lanes 2 and 4 had LDL added. These data are representative of three experiments.

(22,23) and lack of feedback regulation of the LDLr message and LDLr functional protein in colorectal cancers (16) have established a link between regulation of cyclooxygenase to FA metabolites, and a correlation of LDLr expression to cyclooxygenase upregulation. Since submission of this manuscript, two other reports have been published that supported our hypothesis that cyclooxygenase 2 plays a major role in prostate cancer (3,4). The data presented here show that essential fatty acids act like growth factors in stimulating growth of human prostate tumor PC-3 cells. The data also demonstrate that the PC-3 cells lack feedback regulation of LDLr mRNA expression and protein synthesis.

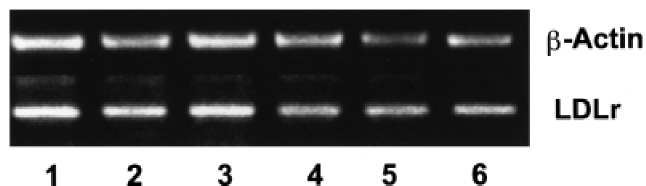

Fig. 6. Effect of FCS and exogenous PGE_2 on *ldlr* gene expression in PC-3 cells. PGE_2 was added to the cells at 4 μg/ml. Expression of LDLr mRNA was measured by RT–PCR. Lane 1, 2% FCS; lane 2, 2% FCS + PGE_2 (3 h); lane 3, 2% FCS + PGE_2 (18 h); lane 4, 10% FCS; lane 5, 10% FCS + PGE_2 (3 h); lane 6, 10% FCS + PGE_2 (18 h). This is representative of two experiments.

Table II. DiI–LDL uptake in PC-3 cells grown in LPDS with or without LDL

Condition	DiI–LDL uptake (fluorescent units)
LPDS	3.10 ± 0.98
LPDS + LDL	2.33 ± 0.81

PC-3 cells were seeded in 96-well plates and grown 24 h in LPDS before measurement of functional LDLr. DiI–LDL in equal molar of LDL was added to both treatment groups for a 30 min incubation, excess label was rinsed off before fluorometric measurement (n = 6).

We demonstrated in this study that LDL increased cell growth and PGE_2 synthesis in PC-3 cells. Since LDL delivers essential fatty acids to the cells, we next examined the effect of essential fatty acids on PC-3 cell growth. When fatty acids, LA, and AA and their product, PGE_2, were added to PC-3 cells, growth was significantly stimulated. Two other fatty acids, OA and EPA did not stimulate growth but significantly retarded growth at higher concentrations. In addition, this finding shows that an increase in available FA energy source is not necessarily the cause of increased growth since media for all cells was supplemented with excess glucose and pyruvate. This finding also suggests a specific role for the essential fatty acids LA and AA. In unpublished data not shown here, non-steroidal anti-inflammatory compounds inhibit cell growth when added with AA or PGE_2 suggesting that AA growth induction may be working through a cyclooxygenase product.

We also examined the effect of increasing concentrations of AA on growth-related gene expression in the PC-3 cell. AA induced c-*fos* within 30 min of addition to the cell in a dose related fashion. Furthermore, AA induces *cox-2* message, which is known to be increased in colorectal and breast cancers (27). This finding directly links essential fatty acids to gene induction of a known tumor marker, cox-2. Although FA derivatives such as δ12-prostaglandin J_2 and 5,8,11,14-eicosatetraynoic acid have been shown to activate the peroxisome proliferator-activated receptors (PPARs), there is no evidence in the literature that LA, AA or PGE_2 directly activate PPARs. It is possible that the up-regulation of c-*fos* or *cox-2* genes is due

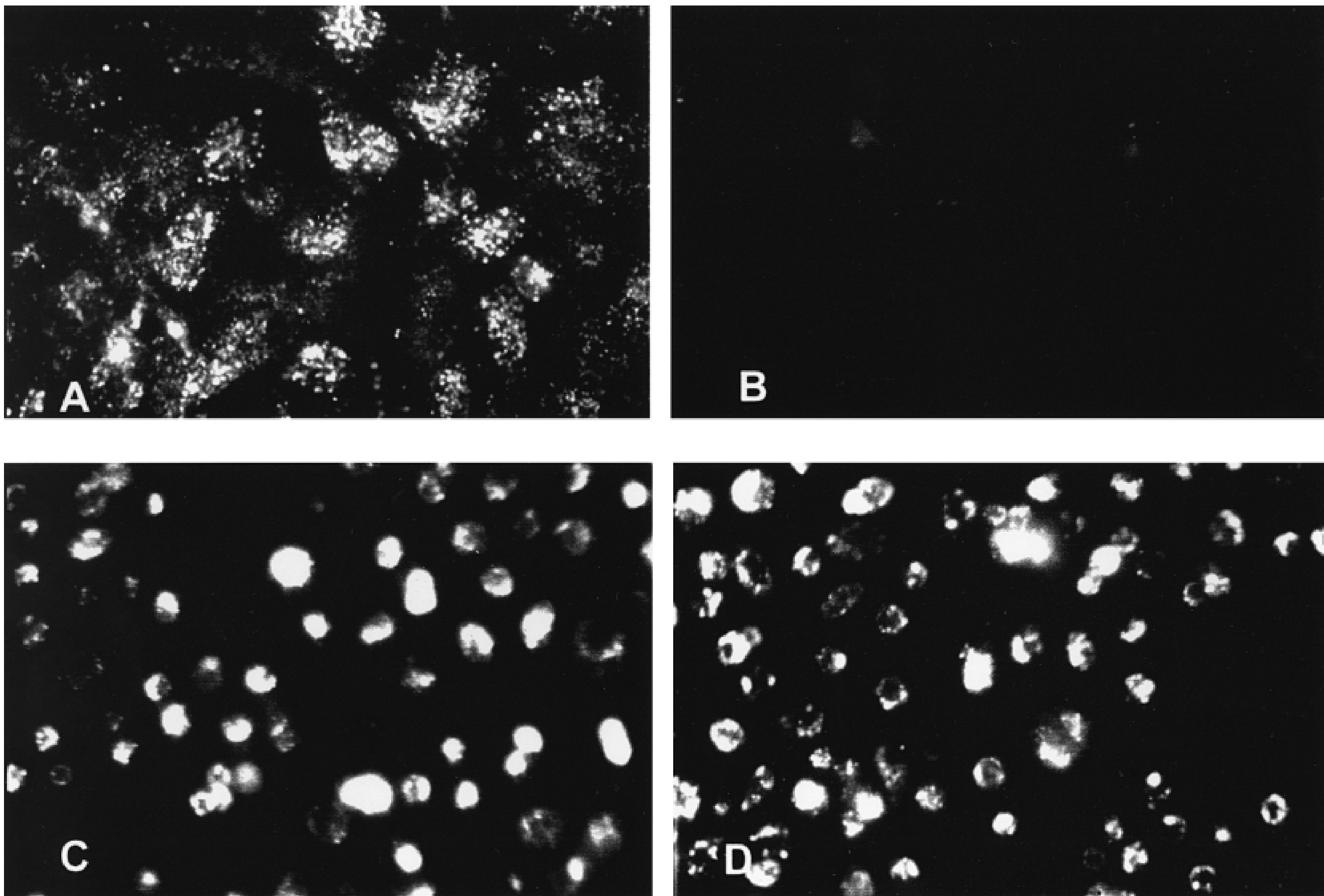

Fig. 7. Visualization of DiI–LDL uptake in normal and tumor cells. Photographs of normal human fibroblasts (A and B) and PC-3 cells (C and D) grown in LPDS ± LDL were taken at 40× magnification. Both cell lines were incubated with equimolar DiI–LDL (10 μg/ml) during the final 30 min. After incubation, unbound DiI–LDL was removed by three washes in PBS with albumin. Photographs were taken with the same exposure time. (**A**) Normal fibroblasts in LPDS; (**B**) normal fibroblasts in LPDS + LDL; (**C**) PC-3 cells in LPDS; (**D**) PC-3 cells in LPDS + LDL.

to PPARs; however, there is only one peroxisome proliferator receptor element (PPRE) consensus, AA(t)C(g)TA(g)GGT(g), which resides approximately –3900 bases upstream of the *cox-2* promoter (28). The CRE element found in the first 200 proximal base pairs of both the c-*fos* and *cox-2* promoter regions is a strong candidate as a key element for up-regulation of message since this laboratory has recently shown that PGE_2 induces CAT reporter with as little as the first proximal 99 base pairs of the promoter, which contains the CRE (29). In addition, recent findings show that prostate tissue and prostate tumors exclusively express the EP4 receptor (23).

Normal regulation of LDLr mRNA expression involves a negative feedback by sterols, which repress the transcription rate of the *ldlr* gene, via the sterol regulatory element (SRE-1) which is composed of three imperfect repeats (30,31). In addition, LDL message is regulated differently in many cells by a variety of hormones or growth factors. For example, transforming growth factor β significantly increases the binding, uptake and degradation of LDL, which is paralleled by an increase in LDLr mRNA levels. Our results presented in Figures 5, 6 and 7 suggest that in PC-3 tumor cells, LDLr mRNA is not regulated by exogenous LDL or by $dmPGE_2$, which is known to stimulate growth and *cox-2* in these cells. This laboratory is currently investigating the regulation of the LDLr in other prostate tumors and tissues, as well as the mechanism that causes the lack of feedback regulation. Preliminary mutation analysis on the PC-3 *ldlr* promoter region –307 to –30 using SSCP did not reveal the presence of a mutation in the *ldlr* promoter region of PC-3 cells (data not shown). In examining other possibilities, although the rate of LDL internalization is similar to that described in other cells (24,25), it is possible that the post-endocytosis processing of the LDLr protein is regulated differently in these cancer cells. Recent discovery of 25-hydroxycholesterol-resistant mutant cells which exhibits constitutive overexpression of *ldlr* and 3-hydroxy-3-methylglutaryl coenzyme A synthase genes has also been reported (32). These cells are unable to suppress transcription of *ldlr* even when cellular sterol levels are greatly elevated. The cells also express a mutant form of SREBP-2 whose sequence is terminated at residue 460, producing a constitutively active factor that is not inhibited by sterols. PC-3 cells may express such a mutant SREBP-2 form, which could explain the lack of regulation of *ldlr* mRNA expression. This possibility is currently under investigation. A publication from this laboratory reports that SREBP-2 is not feedback regulated in prostate tumors (33).

Our finding that cyclooxygenase 2 is upregulated in prostate and other cancers (16,22,23) and the findings of others that cyclooxygenase 2 is upregulated in a majority of prostate cancers (3,4) is in some conflict with the work of Ghosh and Myers (34,35) who reported a prominent role of 5-lipoxygenase in prostate cancers. It is possible that both the cyclooxygenases

and the 5-lipoxygenases are important, since both use LDLr delivered AA as substrates. Moreover, none of the laboratories that studied the role of cyclooxygenases examined lipoxgenases concurrently in the same samples of prostate cancer. An in depth examination of the coordination of both oxygenases is in the planning stages.

The relative effect of ω-3 polyunsaturated fatty acids (PUFA) and ω-6 fatty PUFA has been reported (36,37). In general, the ω-3 PUFAs (EPA, DHA) slow growth, while ω-6 PUFAs (LA, AA) stimulate growth. It is thought that the inhibition of growth by the ω-3 PUFAs is mediated through a decrease in PGE_2 synthesis; this is in agreement with the hypothesis that essential fatty acids stimulate growth through up-regulation of cox-2; synthesis of PGE_2 which in turn acts through its EP4 receptor. In a recent review of the literature on fatty acids and cancer, it was found that ω-6 PUFA are associated with tumors of the breast, colon and prostate. It was also suggested that the ration of dietary ω-3 and ω-6 fatty acids is important to induction of cancer since there is a strong indication that the ω-3 PUFAs have protective action against cancer (38).

The present study shows that both LDL and AA stimulate growth of the PC-3 cells with increased synthesis of PGE_2. EPA and OA do not stimulate growth of tumors. AA caused induction of immediate early genes, c-*fos* and *cox-2* mRNA in a dose-dependent manner. It is probable that the stimulation of immediate early genes and growth is mediated through new synthesis of PGE_2 and stimulation of the EP4 receptor pathway (22,23,33). In addition, PC-3 cells lack normal regulation of LDLr message in the presence of excess exogenous LDL. Since LDL is the primary carrier of physiological AA, a loss of feedback regulation of the LDLr may contribute to uncontrolled tumor growth by increased delivery of excess essential fatty acids.

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