Differential effect of polyunsaturated fatty acids on cell proliferation during human epithelial in vitro carcinogenesis: involvement of epidermal growth factor receptor tyrosine kinase

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Summary Polyunsaturated fatty acids (PUFAs) have been implicated in tumour development and have been shown to influence cell proliferation in vitro. We report here that n-3 and n-6 PUFAs at concentration >10 μM inhibited the proliferation of a human kidney epithelial cell line (2IHKK), which has retained phenotypic characteristics of normal kidney epithelial cells. In contrast, the proliferation was stimulated by n-3 and n-6 PUFAs at concentrations <10 μM under defined growth conditions. The stimulatory effect of n-3 and n-6 PUFAs was even more profound in the presence of EGF. In human kidney epithelial cell lines reflecting different stages of transformation (2IHKK and 1THKE(rain)), the stimulatory effect was abolished both in the presence and absence of EGF. Saturated fatty acids did not show any stimulatory effect on cell growth. The tyrosine kinase inhibitors genistein and tyrphostin-47 inhibited EGF-induced protein tyrosine phosphorylation dose-dependently in the 2IHKK cells, and abolished the growth stimulatory effect of docosahexaenoic acid (DHA). This indicates the involvement of EGF receptor tyrosine kinase activity in the observed increase in cell proliferation.

Keywords: polyunsaturated fatty acid; epidermal growth factor; cell proliferation; in vitro carcinogenesis

Evidence from epidemiological studies suggests that dietary fat may affect the aetiology of cancer, notably breast cancer (Howe et al., 1991) and colorectal cancer (Miller et al., 1983; Nicholson et al., 1988). Polyunsaturated fatty acids (PUFAs) of the n-6 class have in animal models been shown to increase chemically induced tumour development, possibly by affecting mainly post-initiation stages. Also the growth of transplant tumours may be enhanced by n-6 PUFAs (Reddy and Maruyama, 1986; Cave, 1991; Reddy et al., 1991; Rose et al., 1993). In contrast, n-3 PUFAs have frequently been shown to inhibit tumour development in experimental studies (Karmali et al., 1984; O’Connor et al., 1989; de Bravo et al., 1991; Rose and Connolly, 1993; Mehl et al., 1995). This has been supported in a human study on fish diet (rich in n-3 PUFAs) and breast cancer rates (Kaizer et al., 1989). In vitro, the inhibitory effect of PUFAs on cell proliferation has been well documented (Morisaki et al., 1982; Begin et al., 1986; Rose and Connolly, 1991; Hestmark and Lystad, 1992; Krokan et al., 1993; Mehl et al., 1995).

The mechanisms involved in modulation of tumour development by PUFAs are unknown. Lipid peroxidation, however, is considered a major contributing factor (reviewed in Gonzalez, 1992), which is supported by the observation that antioxidants may abolish the growth-inhibitory effect of PUFAs (Hestmark and Lystad, 1992). The differential effect of n-6 and n-3 PUFAs on tumour development has been explained by increased production of peroxidation products with increasing chain length or degree of unsaturation of the fatty acids, correlating grossly with PUFA classes (Begin, 1989; Hestmark and Lystad, 1992; Krokan et al., 1993). In addition, exposure to n-6 PUFAs promotes increased synthesis of arachidonic acid-derived cyclooxygenase- and lipooxygenase-catalysed eicosanoids, which in turn may stimulate tumour cell growth (Carter et al., 1983; Noguchi et al., 1993, 1995). On the contrary, n-3 fatty acids inhibit both the cyclooxygenase and the lipooxygenase pathways (Culp et al., 1979; Corey et al., 1983; Karmali, 1987). Also gene expression and growth factor-mediated signal transduction may be modulated by fatty acids (Tiwari et al., 1991; Distel et al., 1992; Fazio et al., 1992).

The epidermal growth factor (EGF) is involved in growth control of many kinds of cells. EGF binds to the membrane-associated 170 kDa EGF receptor (EGF-R). EGF binding results in receptor dimerisation, thereby activating the intrinsic receptor tyrosine kinase activity, causing its phosphorylation and signal propagation to downstream substrates. Tyrosine kinase activity of the receptor is a prerequisite for EGF-mediated signal transduction (reviewed in Ullrich and Schlessinger, 1990; van der Geer and Hunter, 1994). EGF-mediated signal transduction may be modulated by PUFAs (Bandyopadhyay et al., 1987, 1993; Casabell et al., 1991; Glasgow et al., 1992).

Recently, we reported a correlation between sensitivity among different cell lines to the growth-inhibitory effect of PUFAs in vitro and the ability of these fatty acids to reduce tumour growth rates in vivo (Krokan et al., 1993; Mehl et al., 1995). Little is known about the role of PUFAs on growth factor-mediated signal transduction during in vitro carcinogenesis, especially fatty acids of the n-3 class. We have developed an in vitro human multistep model suitable for human epithelial carcinogenesis studies (Tveit et al 1989; Haugen et al., 1990; Mollerup et al., 1996). The present study was undertaken to investigate the influence of n-3 [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] and n-6 [linoleic acid (LA) and arachidonic acid (ARA)] PUFAs on EGF-mediated growth control during human epithelial in vitro carcinogenesis.

Materials and methods

Chemicals

Dulbecco’s modified Eagle Medium (DMEM)/F12 (1:1), EGF, insulin, transferrin, hydrocortisone, sodium-selenite, palmitic acid, stearic acid, linoleic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, fatty acid-free bovine serum albumin (BSA) (fraction V) and genistin were all purchased from Sigma Chemical Company, St Louis, MO, USA. Tyrphostin-47 (3,4-dihydroxy-a-cyanohithiocinnama- mide) was from Fluka Chemie AG, Buchs, Switzerland. Fetal calf serum (FCS) was from Gibco BRL and [3H]thymidine was from NEN Research Products, Du Pont de Nemour & Co., Boston, MA, USA.
Cell lines and culture conditions

Immortalisation of human kidney epithelial cells by exposure to Ni²⁺ (1IHKE), and the subsequent transformation by v-Ha-ras transfection (1THKEras) has been described previously (Tveito et al., 1989; Haugen et al., 1990; Mahle et al., 1992). Recently, we have repeated the nickel-exposure experiment and established a cell line (2IHKE) with several properties of normal kidney epithelial cells. This cell line exhibits EGF- and anchorage-dependence (unpublished results). The kidney epithelial cell lines were cultured in DMEM/F12 (1:1) medium, supplemented with EGF (10 ng ml⁻¹), insulin (5 µg ml⁻¹), transferrin (5 mg ml⁻¹), hydrocortisone (36 ng ml⁻¹), sodium selenite (5 ng ml⁻¹) and 1% (1IHKE and 1THKEras) or 5% FCS (2IHKE). The cell lines were maintained at 37°C in humidified air containing 5% carbon dioxide.

Stock solutions of fatty acids [free fatty acids: palmitic acid (PA, C16:0), stearic acid (SA, C18:0), linoleic acid (LA, C18:2, n-6), arachidonic acid (ARA, C20:4, n-6), eicosapentaenoic acid (EPA, C20:5, n-3), and docosahexaenoic acid (DHA, C22:6, n-3)] were prepared in 99% ethanol, and stored at −70°C under nitrogen for no longer than a month. After addition of fatty acids, the medium was incubated for 1 h at 37°C in air containing 5% carbon dioxide, before addition to cell cultures. Tyrosine kinase inhibitors (dissolved in dimethyl sulfoxide) were added to cell cultures 1 h before addition of fatty acids and EGF, and were present throughout the incubation period.

DNA synthesis

The proliferative activity of the cells was measured by estimating [³H]thymidine ([³H]Tdr) incorporation into DNA, essentially as previously described (Mollerup et al., 1996). Cells were seeded in 24-well trays, at a density of 1–5 × 10⁴ cells per well in DMEM/F12 medium supplemented with FCS. Two days later the medium was replaced with DMEM/F12 supplemented with 2.25 mg ml⁻¹ fatty acid-free BSA (fraction V) (serum-free medium, SFM) and the indicated concentrations of fatty acids or tyrosine kinase inhibitors (genistein or tyrophostin-47). [³H]Tdr (2.5 mCi ml⁻¹, 1 mCi per well, 82.5 Ci mmol⁻¹ specific activity) was added 48 h later, and incubation continued for 4 h at 37°C. Cells were then fixed in ice-cold methanol for 20 min, followed by three washes in Hepes-buffered saline. Unincorporated [³H]Tdr was extracted from the cells by incubation in trichloroacetic acid (5%, w/v) for 20 min at 4°C followed by three washes in water. Cells were solubilised in 0.5% sodium dodecyl sulphate (SDS), 0.25 M sodium hydroxide at 60°C for 20 min. Cell lysates were transferred to scintillation vials and radioactivity was measured by liquid scintillation counting. P<0.05, Student’s t-test, was considered statistically significant. Experiments were repeated several times with similar results. Parallel experiments with measurement of cell proliferation by cell counting gave similar results (data not shown).

Immunoblotting

Cells were seeded in 35 mm dishes as a density of 0.75–1.5 × 10⁵ cells per dish in DMEM/F12 and treated with tyrosine kinase inhibitors as indicated above. Cells were then exposed to 200 ng ml⁻¹ EGF for 5 min at 37°C. The dishes were washed three times in ice-cold Hepes-buffered saline, and cellular proteins were solubilised in SDS-PAGE sample buffer and denatured at 95°C for 5 min (Laemmli, 1970). Parallel dishes were seeded for determination of protein content by the method of Lowry et al. (1951).

Proteins were separated on 7.5% SDS-PAGE mini gels (Bio Rad Mini-Protean II, Bio-Rad Laboratories, Richmond, CA, USA) and transferred to Immobilon-P PVDF membranes (Millipore Corporation, Bedford, MA, USA). Tyrosine-phosphorylated proteins were detected with a polyclonal phosphotyrosine-specific antibody (Mollerup et al., 1996), followed by a secondary horseradish peroxidase-linked antibody, and visualised by the enhanced chemiluminescence detection kit (Amersham International, UK) and exposure of autoradiography film (Kodak X-OMAT S). Densitometric analysis of autoradiography films was performed on a Macintosh 7100/66AV computer using the public domain NIH Image program.

Results

DNA synthesis in human kidney epithelial cell lines—response to n-3 PUFAs

We have previously shown that n-3 and n-6 PUFAs inhibited in vitro proliferation of human kidney epithelial cell lines in a dose-dependent manner (Mahle et al., 1995). These experiments were carried out in serum-containing culture media. In order to investigate further the effect of polyunsaturated fatty acids on cell proliferation in these cell lines, experiments were performed. The effect of n-3 PUFAs on cell proliferation is shown in Figure 1. The results were expressed as counts per minute (cpm) in triplicate wells. There was a dose-dependent inhibition of [³H]Tdr incorporation in all cell lines exposed to n-3 PUFAs. The results are representative of three independent experiments.

Figure 1 Effect of n-3 PUFAs on [³H]thymidine incorporation in 2IHKE, 1IHKE and 1THKEras cells. Cells were exposed to increasing concentrations of EPA or DHA with and without EGF in SFM for 48 h. [³H]TDR uptake was measured as described in Materials and methods. Each point corresponds to the mean of three estimations of acid-precipitable [³H] (cpm) at each concentration of fatty acid. Error bars denote s.d. (a) 2IHKE, (b) 1IHKE and (c) 1THKEras cells. (D) EGF without EFA; (E) EPA + 10 ng ml⁻¹ EGF; (F) DHA without EGF, and (G) DHA + 10 ng ml⁻¹ EGF.
carried out under defined conditions. Figure 1a shows the result of a [\(^3\)H]thymidine incorporation experiment with 2IHKER cells incubated in the presence of increasing concentrations of EPA and DHA w/w EGF. The 2IHKER cell line showed a dual response to the n-3 PUFAs. In addition to the observed growth inhibition at concentrations >10 \(\mu\)M, EPA and DHA stimulated proliferation of 2IHKER cells significantly at low concentrations (1–5 \(\mu\)M) in the absence of EGF. In the presence of EGF, the growth stimulation was even more profound with significantly increased [\(^3\)H]TdR incorporation at 1–5 \(\mu\)M EPA and 1–10 \(\mu\)M DHA, indicating a synergistic effect of n-3 PUFAs and EGF on cell proliferation. The fatty acids inhibited growth at concentrations >10 \(\mu\)M in the presence of EGF. Similar results were obtained with normal adult human kidney epithelial cells (data not shown).

1IHKER cells, an immortalised cell line that has abrogated normal growth regulation in respect of EGF (Mollerup et al., 1996), were growth inhibited at high concentrations of n-3 PUFAs either in the absence or presence of EGF (Figure 1b). Tumorigenic 1THKERas cells were only slightly inhibited by the fatty acids (Figure 1c). However, in both the 1IHKER and 1THKERas cell lines, no stimulatory effect of EPA and DHA on [\(^3\)H]TdR incorporation was observed, indicating that this effect was abrogated during in vitro carcinogenesis.

**Involvement of EGF receptor tyrosine kinase in n-3 PUFA stimulation of cell proliferation**

The results from Figure 1 indicated a synergistic effect of n-3 PUFAs and EGF on cell proliferation. To study the mechanism(s) involved, the effect of specific inhibitors of tyrosine kinase activity was investigated. 2IHKER cells were incubated in the presence of tyrosine kinase inhibitors at similar conditions to Figure 1. Cells were then exposed to EGF for 5 min. Cellular extracts were subjected to immunoblotting and probed with a phosphotyrosine-specific antibody. As shown in Figure 2, a 5 min EGF pulse resulted in tyrosine phosphorylation on the EGF-R and other cellular proteins. Genistein, which is a general inhibitor of tyrosine kinases (Akiyama et al., 1987), and the relatively specific inhibitor of EGF-R tyrosine kinase activity, tyrphostin-47 (Gazit et al., 1989), inhibited EGF-induced tyrosine phosphorylation dose-dependently. Genistein was a more

**Figure 3** Effect of (a) genistein and (b) tyrphostin-47 on DHA-stimulated [\(^3\)H]thymidine incorporation in 2IHKER cells. Cells were exposed to DHA in SFM for 48 h with and without EGF, and with and without genistein or tyrphostin-47. [\(^3\)H]TdR uptake (c.p.m.) was measured as in Figure 1. Open symbols denote exposure without EGF and closed symbols with 10 ng ml\(^{-1}\) EGF. In (a), (○ and ■) without, and (▲ and ▼) with 25 \(\mu\)M genistein, and in (b), (□ and ■) without, and (△ and ▲) with 50 \(\mu\)M tyrphostin-47.

**Figure 2** Effect of (a) genistein and (b) tyrphostin-47 on EGF-induced tyrosine phosphorylation in 2IHKER cells. Cells were incubated in the presence of genistein or tyrphostin-47 for 48 h at the indicated concentrations. Cells were then exposed to EGF (200 ng ml\(^{-1}\) for 5 min). Cellular extracts were subjected to immunoblotting with a phosphotyrosine-specific antibody (see Materials and methods). EGF-R denotes position of EGF receptor in immunoblots with an anti-EGF receptor antibody.
potent inhibitor of tyrosine kinase activity than tyrphostin-47. The effect of the inhibitors on DHA-stimulated proliferation of 2IHKE cells was studied. Genistein, at a concentration of 25 μM, inhibited proliferation and totally abolished the stimulatory effect of DHA in the 2IHKE cells, both in the absence and presence of EGF (Figure 3a). Under similar conditions, tyrphostin-47, at a concentration of 50 μM, also abolished stimulation of DNA synthesis by DHA, as shown in Figure 3b (tyrphostin-47 was not as inhibitory to [3H]TdT incorporation as genistein). Densitometric scanning of the band corresponding to the EGF-R in Figure 2 revealed that the phosphotyrosine content at 25 μM genistein and 50 μM tyrphostin-47 was reduced to 32% and 43% of the controls respectively. Together, these data indicate the involvement of EGF receptor tyrosine kinase in the n-3 PUFA-induced stimulation of 2IHKE cell proliferation.

n-6 PUFA s exert an effect similar to n-3 PUFA s on DNA synthesis in 2IHKE cells

The n-6 PUFA s LA and ARA were administered to 2IHKE cells under defined conditions similar to the n-3 fatty acid experiment (Figure 4). Low concentrations of either LA or ARA stimulated proliferation. In the absence of EGF, [3H]TdT incorporation was significantly increased at 1–5 μM LA and 1–2.5 μM ARA respectively. Again, a synergistic effect of EGF was observed. In the case of LA and EGF a broader stimulatory concentration range (1–20 μM) was demonstrated, whereas [3H]TdT incorporation was significantly increased at 1–2.5 μM ARA in the presence of EGF. As in the case of the n-3 PUFA s, high concentrations of LA or ARA inhibited cell proliferation.

Saturated fatty acids

In contrast to the n-3 and n-6 PUFA s, the saturated fatty acids palmitic and stearic acid did not stimulate proliferation of 2IHKE cells at any concentrations tested (Figure 5). Rather, these fatty acids inhibited cell proliferation in a dose-dependent manner, PA being more inhibitory than SA.

Discussion

We report here that low concentrations of n-3 PUFA s stimulated proliferation of the 2IHKE cell line under defined growth conditions. In the presence of EGF, the growth-stimulatory effect of PUFA s was even more profound. Specific tyrosine kinase inhibitors [genistein and tyrphostin-47 (Akiyama et al., 1987; Gazit et al., 1989)] inhibited EGF-induced protein tyrosine phosphorylation. In addition, genistein and tyrphostin-47 totally abrogated the growth-stimulatory effect of DHA, both in the absence or presence of EGF, suggesting interaction with tyrosine kinase signal transduction pathways especially involving the EGF-R. This is in agreement with the reported findings that an EGF-R-blocking antibody caused suppression of LA-stimulated proliferation of a human prostate cancer cell line (DU145M) in serum-free medium (Connolly and Rose, 1992). The possibility that other factors involved in the growth control of the 2IHKE cells may be affected by genistein and tyrphostin-47, however, cannot be excluded. n-6 and n-9 PUFA s have been reported to inhibit PDGF receptor tyrosine kinase activity in both intact cells and membrane preparations (Tomaska and Resnick, 1993). Treatment of the 2IHKE cells with n-3 PUFA s at growth-stimulatory conditions (in the absence of EGF) did not affect the EGF-induced level of cellular tyrosine phosphorylation (data not shown).

PUFA s have been shown to modulate EGF-mediated signal transduction (Bandyopadhyay et al., 1987, 1993; Casabieli et al., 1991). These studies included fatty acids of the n-6, n-7 and n-9 classes. However, studies on the action of n-3 PUFA s on signal transduction are limited, although modulation of the catalytic activity of protein kinase C and type I CAM- dependent protein kinase has been demonstrated (Speizer et al., 1991).

Interestingly, we observed a similar growth-stimulatory effect of low concentrations of both n-3 and n-6 PUFA s on 2IHKE cells, which have retained several phenotypical features of normal cells. Studies have shown that human prostate (PC-3) and breast (MDA-MB-231) cancer cell lines were growth stimulated by low concentrations of n-6 PUFA (LA) under defined conditions. However, in contrast to our results low concentrations of the n-3 PUFA s, EPA and DHA were growth inhibitory (Rose and Connolly, 1990, 1991). LA and ARA also stimulated cell proliferation of murine colon adenocarcinomas (Hussey and Tisdale, 1994). Perfusion of Morris hepatomas (7288C7Tc) in situ with donor whole blood containing added PUFA s resulted in an increased (n-6) or decreased (n-3) rate of DNA synthesis (Sauer and Dauchy, 1992). The increase in DNA synthesis rate was observed at high plasma concentrations of n-6 PUFA s (200–600 μM). In vivo studies have shown that PUFA s of the n-6 class may enhance both chemically induced and transplanted tumour development and that PUFA s of the n-3 class frequently exert an opposite effect, inhibiting tumour development (Roebuck et al., 1981; Karmali et al., 1984; Braden and Carroll, 1986; BORGeson et al., 1989; Rose et al., 1993; Mæhle et al., 1995). The in vivo differences have been
explained by differences in eicosanoid synthesis and different lipid peroxidation potential (Gonzalez et al., 1993; Noguchi et al., 1995). Our short-term studies on growth-regulatory control by PUFA's does not account for long-term in vivo effects, and a clear correlation between in vivo and in vitro data can therefore not be expected.

The stimulatory effect of the n-3 and n-6 PUFA's was confined to the 2HKE cell line, which shows apparent normal EGF-mediated growth-regulatory control (unpublished data). In general, PUFA's have been implicated in promotion stages of carcinogenesis (Aylsworth et al., 1984; Reddy et al., 1991; Ronai et al., 1991). Our data on the action of low concentrations of PUFA's are consistent with a multistep carcinogenesis model where stimulation of clonogenic growth of initiated cells (2HKE) will enhance the possibility of a hit by secondary carcinogens, and this in turn may result in tumorigenic conversion. The growth-stimulatory effect of PUFA's correlates with normal EGF growth-regulatory control. In our in vitro model, the 1HKE and 1THKeraz cell lines showed a marked loss of stimulatory response to low concentrations of PUFA's, indicating an alteration taking place during the in vitro carcinogenic process. These results correlate with our previous observations that the 1HKE and 1THKeraz cell lines have abrogated normal growth-regulatory control in respect of mitogenic response to EGF and expression of the EGF-R (Mollerup et al., 1996). The proliferation of normal cells is a highly regulated process, controlled by the interplay of growth-inducing and growth-inhibitory signals. Although normal human kidney epithelial cells are stimulated by PUFA's in vitro (data not shown), this characteristic might not be expressed in vivo, indicating no conflict with the perception of PUFA's functioning as tumour promoters.

Using defined growth conditions, we observed an increased resistance towards the growth-inhibitory effect of high concentrations of DHA with increased transformation/malignant potential of the human kidney epithelial cell lines (2HKE < 1HKE < 1THKeraz). Sensitivity to the growth-inhibitory effect of PUFA's varies considerably between different cell types (Krokkan et al., 1993; Mahle et al., 1995). In contrast to the data presented here, it has been reported that normal cells may be more resistant to the inhibitory effect than tumour cells in vitro (Begin et al., 1986; Krokkan et al., 1993), and it might be expected that sensitivity should increase during development of the transformed phenotype within cell lines with a common origin.

In conclusion, we have shown that n-3 and n-6 fatty acids may stimulate the proliferation of immortalised human kidney epithelial cells with apparent normal EGF growth-regulatory control. This effect apparently involves EGF-R tyrosine kinase activity. Furthermore, our results demonstrate that the stimulatory growth response to the PUFA's was abrogated during in vitro transformation of the cell lines. Further studies are needed to determine the specific mechanism(s) involved.

**References**

AKIYAMA T, ISHIDA J, NAKAGAWA S, OGAWARA H, WATANABE S-I, ITOH N, SHIBUYA M AND FUKAMI Y. (1987). Genistein, a specific inhibitor of tyrosine-specific protein kinases. J. Biol. Chem., 262, 5592–5595.

AYLSWORTH CF, JONE C, TROSKO JE, MEITES J AND WELCH CW (1984). Promotion of 7,12-dimethylbenz(a)anthracene-induced mammary tumorigenesis by high dietary fat in the rat: possible role of intercellular communication. J. Natl Cancer Inst., 72, 637–645.

BANDYOPADHYAY GK, IMAGAWA W, WALLACE D AND NANDI S. (1987). Linoleate metabolites enhance the in vitro proliferative response of mouse mammary epithelial cells to epidermal growth factor. J. Biol. Chem., 262, 2750–2756.

BANDYOPADHYAY GK, HWANG S-I, IMAGAWA W AND NANDI S. (1993). Role of polyunsaturated fatty acids as signal transducers: Amplification of signals from growth factor receptors by fatty acids in mammary epithelial cells. Prost. Leuk. Ess. Fatty Acids, 48, 71–78.

BEGIN ME. (1989). Tumor cytotoxicity of essential fatty acids. Nutrition, 5, 258–260.

BEGIN ME, ELLS G, DAS UN AND HORKORIN DF. (1986). Differential killing of human carcinoma cells supplemented with n-3 and n-6 polynsaturated fatty acids. J. Natl Cancer Inst., 77, 1053–1062.

BORGESON CE, PARDINI L, PARDINI RS AND REITZ RC. (1989). Effects of dietary fish oil on human mammary carcinoma and on lipid-metabolizing enzymes. Lipids, 24, 290–295.

BRADEN LM AND CARROLL KK. (1966). Dietary polyunsaturated fat in relation mammary carcinosign in rat. Lipids, 21, 285–288.

CARTER CA, MILHOLLAND RJ, SHEA W AND IP MM. (1983). Effect of the prostaglandin synthase inhibitor indomethacin on 7,12-dimethylbenz(a)anthracene-induced mammary tumorigenesis in rats fed different levels of fat. Cancer Res., 43, 3559–3562.

CASABIELL X, PANDELLA A AND CASANUEVA FF. (1991). Regulation of epidermal-growth-factor-receptor signal transduction by cis-unsaturated fatty acids. Evidence for a protein kinase C-independent mechanism. Biochem. J., 287, 679–687.

CAVE WT. (1991). Dietary n-3 (ω-3) polynsaturated fatty acid effects on animal tumorigenesis. FASEB J., 5, 2160–2166.

CONNOLLY JM AND ROSE DP. (1992). Interaction between epidermal growth factor-mediated autocrine regulation and linoleic acid-stimulated growth of a human prostate cancer cell line. Prostate, 20, 151–158.

COREY EJ, SHIH C AND CASHMAN JR. (1983). Docosahexaenoic acid is a strong inhibitor of prostaglandin but not leukotriene biosynthesis. Proc. Natl Acad. Sci. USA, 80, 3581–3584.

CULP BR, TITUS BJ AND LANDS WE. (1979). Inhibition of prostaglandin biosynthesis by eicosapentaenoic acid. Prostaglandins Med., 3, 269–278.

DE BRAVO MG, DE ANTUENO RJ, TOLEDO J, DE TOMAS ME, MERCURI OF AND QUINTANS C. (1991). Effects of an eicosapentaenoic and docasahexaenoic acid concentrate on a human lung carcinoma grown in nude mice. Lipids, 26, 866–870.

DISTEL RJ, ROBINSON GS AND SPIEGELMAN BM. (1992). Fatty acid regulation of gene expression. Transcriptional and post-transcriptional mechanisms. J. Biol. Chem., 267, 5937–5941.

FAZIO YM, BARRERA G, MARTINOTTI S, FARAGE MG, GIGLIONI B, FRATI L, MANZARI V AND DIANZANI MU. (1992). 4-Hydroxynonenal, a product of cellular lipid peroxidation, which modulates c-myc and globin gene expression in K562 erythroleukemic cells. Cancer Res., 52, 4866–4871.

GAZIT A, YAISH P, GIolon G AND LEVITZKI A. (1989). Tyrophostins I: synthesis and biological activity of protein tyrosine kinase inhibitors. J. Med. Chem., 32, 2344–2352.

**Abbreviations**

EGF, epidermal growth factor; EGF-R, epidermal growth factor receptor; PA, palmitic acid (C16:0); SA, stearic acid (C18:0); LA, linoleic acid (C18:2, n-6); ARA, arachidonic acid (C20:4, n-6); EPA, eicosapentaenoic acid (C20:5, n-3); DHA, docosahexaenoic acid (C22:6, n-3); PUFA, polyunsaturated fatty acid; Tr, thymidine.

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GLASGOW WC, AFSHARI CA, BARRETT JC AND ELING TE. (1992). Modulation of the epidermal growth factor mitogenic response by metabolites of linoleic and arachidonic acid in Syrian hamster embryo fibroblasts. *J. Biol. Chem.*, 267, 10771–10779.

GONZALEZ MJ, SCHEMMLER RA, DUGAN L-R JR, GRAY JI AND WELSH CW. (1993). Dietary fish oil inhibits human breast carcinoma growth: a function of increased lipid peroxidation. *Breast Cancer Res. Treat.*, 28, 257–273.

HAUGEN A, RYBERG D, HANSTEEN I-L AND AMSTAD P. (1990). Neoplastic transformation of a human kidney epithelial cell line with γ-Ha-ras oncogene. *Int. J. Cancer*, 45, 572–577.

HUSSEY HJ AND TISDALE MJ. (1994). Effect of polyunsaturated fatty acids on the growth of murine colon adenocarcinomas in vivo and in vitro. *Br. J. Cancer*, 70, 6–10.

HOSTMARK AT AND LYSTAD E. (1992). Growth inhibition of human hepatoma cells (HepG2) by polyunsaturated fatty acids. Protection by albumin and vitamin E. *Acta Physiol. Scand.*, 144, 83–88.

KAIZER NF, BOYD NF, KRIKUOV V AND TRITCHLER D. (1989). Fish consumption and breast cancer risk: an ecological study. *Nutr. Cancer, 12*, 61–83.

KARMALLA R, MARSH J AND FUCHS C. (1984). Effect of omega-3 fatty acids on growth of a rat mammary tumor. *J. Nutr. Cancer*, 73, 457–461.

KARMALLA R. (1987). Eicosanoids in neoplasia. *Prev. Med.*, 16, 493–502.

KROKAN HE, RUDRA PK, SCHØBERG S, SLETTAHLJEW W, SOLUM K, MOLLERUP S, EIERTSEN E, MÆHLE L AND HAUGEN A. (1992). Effect of n-3 fatty acids on the growth of human tumor cell lines in culture and in nude mice. *In Omega-3 Fatty Acids: Metabolism and Biological Effects*. Drevon CA, Bakkaas I and Krokkan HE. (eds) pp. 327–334. Birkhäuser Verlag: Basle.

LAEMMLI UK. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

LOWRY OH, ROSEBROUGH NJ, FARR AL AND RANDALL RJ. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193, 265–275.

MÆHLE L, METCALF RA, RYBERG D, BENNETT WP, HARRIS CC AND HAUGEN A. (1992). Altered p53 gene structure and expression in human epithelial cells after exposure to nickel. *Cancer Res.*, 52, 218–221.

MÆHLE L, EIERTSEN E, MOLLERUP S, SCHØBERG S, KROKAN HE AND HAUGEN A. (1995). Effect of n-3 fatty acids during neoplastic progression and comparison of in vitro and in vivo sensitivity of two human tumor cell lines. *Br. J. Cancer*, 71, 691–696.

MILLER AB, HOWE GR, JAIN M, CRAIB KJP AND HARRISON L. (1983). Food items and food groups as risk factors in a case–control study of diet and colorectal cancer. *Int. J. Cancer*, 32, 155–161.

MOLLERUP S, RIVEDAL E, MÆHLE L AND HAUGEN A. (1996). Nickel(II) induces alterations in EGF- and TFG-β1-mediated growth control during malignant transformation of human kidney epithelial cells. *Carcinogenesis*, 17, 361–367.

MORISAKI N, SPRECHER H, MILO GE AND COWERNELL DG. (1992). Fatty acid specificity in the inhibition of cell proliferation and its relationship to lipid peroxidation and prostaglandin biosynthesis. *Lipids*, 17, 893–899.

NICHOLSON ML, NEOPTOLEMOS JP, CLAYTON HA AND HEGARTY AM. (1988). Diet and colorectal cancer. *Int. Clin. Nutr. Rev.*, 8, 180–197.

NOGUCHI M, KITAGAWA H, MIYAZAKI I AND MIJUKAWA Y. (1993). Influence of esculetin on incidence, proliferation, and cell kinetics of mammary carcinomas induced by 7,12-dimethylbenz[a]anthracene in rats on high- and low-fat diets. *Jpn. J. Cancer Res.*, 84, 1014–1014.

NOGUCHI M, ROSE DP, EARHARD M AND MIYAZAKI I. (1995). The role of fatty acids and eicosanoid synthesis inhibitors in breast carcinoma. *Oncoology*, 52, 265–271.

O’CONNOR TP, ROEBUCK BD, PETERSON FJ, LOKESH B, KINSELLJA AND CAMPELL TC. (1989). Effect of dietary omega-3 and omega-6 fatty acids on development of azasaterine-induced preneoplastic lesions in rat pancreas. *J. Natl Cancer Inst.*, 81, 585–586.

REDDY BS AND MARUYAMA H. (1986). Effect of different levels of dietary corn oil and lard during the initiation phase of colon carcinogenesis in F344 rats. *J. Natl Cancer Inst.*, 77, 815–822.

REDDY BS, BURILL C AND RIGOTTI J. (1991). Effects of diets high in ω-3 and ω-6 fatty acids on initiation and postinitiation stages of colon carcinogenesis. *Cancer Res.*, 51, 487–491.

ROEBUCK BD, YAGER JD, LONGNECKER DS AND WILPONE DA. (1981). Promotion by unsaturated fat of azaserine-induced pancreatic carcinogenesis in the rat. *Cancer Res.*, 41, 3961–3966.

RONAI Z, LAU Y AND COHEN LA. (1991). Dietary N-3 fatty acids do not affect induction of Ha-ras mutations in mammary glands of NMU-treated rats. *Mol. Carcinogen.*, 4, 120–128.

ROE DP AND CONNOLLY JM. (1990). Effects of fatty acids and inhibitors of eicosanoid synthesis on the growth of a human breast cancer cell line in culture. *Cancer Res.*, 50, 7139–7144.

ROE DP AND CONNOLLY JM. (1991). Effects of fatty acids and eicosanoid synthesis inhibitors on the growth of two human prostate cancer cell lines. *Prostate*, 18, 243–254.

ROE DP AND CONNOLLY JM. (1993). Effects of dietary omega-3 fatty acids on human breast cancer growth and metastases in nude mice. *J. Natl Cancer Inst.*, 85, 1743–1747.

ROE DP, HATA LA, AND DOLLY JM AND RAYBURN J. (1993). Effect of diets containing different levels of linoleic acid on human breast cancer growth and lung metastasis in nude mice. *Cancer Res.*, 53, 4686–4690.

SAUER LA AND DAUCHY RT. (1992). The effect of omega-6 and omega-3 fatty acids on 1H-thymidine incorporation in hepatoma 728CCTC perfused in situ. *Br. J. Cancer*, 66, 297–303.

SPEIZER LA, WATSON MJ AND BRUNTON LL. (1991). Differential effects of omega-3 fish oils on protein kinase activities in vitro. *Am. J. Physiol.*, 261, E109–E114.

TIWARI RK, MUKHOPADHYAY B, TELANG NT AND OSBORNE MP. (1991). Modulation of gene expression by selected fatty acids in human breast cancer cells. *Anticancer Res.*, 11, 1383–1388.

TOMASKA I AND RESNIK CI. (1993). Suppression of platelet-derived growth factor receptor tyrosine kinase activity by unsaturated fatty acids. *J. Biol. Chem.*, 268, 5317–5322.

TVEITO G, HANSTEEN I-L, DALEN H AND HAUGEN A. (1989). Immunostaining of normal human kidney epithelial cells by nickel(II). *Cancer Res.*, 49, 1829–1835.

ULLRICH A AND SCHLESSINGER J. (1990). Signal transduction by receptors with tyrosine kinase activity. *Cell*, 61, 203–212.

VAN DER GEER P AND HUNTER T. (1994). Receptor protein-tyrosine kinases and their signal transduction pathways. *Ann. Rev. Cell Biol.*, 10, 251–337.