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RESEARCH PAPER

Modulation of gene expression in eicosapentaenoic acid and docosahexaenoic acid treated human colon adenoma cells

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Abstract Epidemiological studies suggest that high fish intake is associated with a decreased risk of colorectal cancer which has been linked to the high content of the *n*-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acids (EPA) and docosahexaenoic acid (DHA) in some fish. The aim of the study was to compare the modulation of gene expression in LT97 colon adenoma cells in response to EPA and DHA treatment. Therefore, we used custom-designed cDNA arrays containing probes for 306 genes related to stress response, apoptosis and carcinogenesis and hybridised them with cDNA from LT97 cells which were treated for 10 or 24 h with 50 μM EPA or DHA. There was a marked influence of *n*-3 PUFA on the expression of several gene types, such as detoxification, cell cycle control, signaling pathways, apoptosis and inflammation. DHA and EPA generally modulated different sets of genes, although a few common effects were noted. In our approach, we used preneoplastic adenoma cells which are a relevant model for target cells of chemoprevention. If verified with real time PCR, these results identify genes and targets for chemoprevention of colon cancer.

Keywords cDNA array · *n*-3 polyunsaturated fatty acids · Colon cancer · Gene expression

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Short communication

Epidemiological studies suggest that high fish intake is associated with a decreased risk of colorectal cancer (2, 3, 7). Most of the beneficial effects have been linked to the high content of the *n*-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in some fish. EPA and DHA are essential for humans and found most notably in oily fish like salmon or herring. The intake of *n*-3 PUFAs in the world differs and is in direct relation to the relative fish consumption. The incidence of colorectal cancer in countries with high fish consumption such as Greenland or Japan is reported to be lower (9). Also, in vivo studies in rats have shown antitumourigenic effects of a diet rich in fish oil compared to other oils, such as corn oil (5). Evidence suggests that fish-related compounds act post-initiation to reduce transition of adenomas to tumours. However, the molecular mechanisms behind the anticancer effects of a diet rich in fish are not yet clarified.

The aim of the present study was to examine the modulation of expression of 306 genes related to stress response, apoptosis and carcinogenesis by comparing the effects of EPA and DHA. Human colon adenoma cells were chosen since they are a model for target cells for chemoprevention in vivo. Previous in vitro studies in this field have used cancer cell lines such as HT29 (1) or Caco-2 (6) rather than cells derived from adenomas, for example LT97.

Materials and methods

Gene expression studies were performed using LT97 human colon adenoma cells (4, 8) treated with a non-cytotoxic

Table 1 Summary of selected genes that are modulated by treatment of LT97 human colon adenoma cells with 50 μM EPA ($n = 3$) or DHA ($n = 2$)

Accession	Gene name	Gene symbol	FC	SD	p value (t test)
EPA 10 h					
NM_001228	Caspase 8	CASP8	0.10	0.17	0.17
NM_001074	UDP glucuronosyltransferase 2 family, polypeptide B7, 8, 10, 11	UGT2B7_8_10_11	0.23	0.39	0.39
NM_000410	Hemochromatosis	HFE_3	0.25	0.43	0.37
NM_000578	Solute carrier family 11, member 1	SLC11A1	0.29	0.50	0.92
NM_030753	Wingless-type MMTV integration site family, member 3	WNT3	0.30	0.53	0.95
NM_022467	Carbohydrate sulfotransferase 8	CHST8	2.02	2.66	0.54
NM_000854	Glutathione S-transferase theta 2	GSTT2	4.04	3.71	0.31
EPA 24 h					
NM_000463	UDP glucuronosyltransferase 1 family, polypeptide A1	UGT1A1	2.00	1.06	0.29
NM_004985	v-Ki-ras2 kirsten rat sarcoma viral oncogene homolog	KRAS2A-B	2.02	0.31	0.26
NM_002502	Nuclear factor of kappa light polypeptide gene enhancer in B cells 2	NFKB2	2.12	0.92	0.41
NM_003998	Nuclear factor of kappa light polypeptide gene enhancer in B cells 1	NFKB1	2.16	1.08	0.19
NM_000854	Glutathione S-transferase theta 2	GSTT2	2.41	1.65	0.46
NM_000178	Glutathione synthetase	GSS	2.54	2.31	0.30
NM_001461	Flavin containing monooxygenase 5	FMO5	2.58	0.54	0.15
DHA 10 h					
NM_001228	Caspase 8	CASP8	0.15	0.21	0.80
NM_000617	Solute carrier family 11, member 2	SLC11A2_2	0.27	0.16	0.30
NM_000410	Haemochromatosis	HFE_3	0.28	0.37	0.41
NM_001074	UDP glucuronosyltransferase 2 family, polypeptide B7, 8, 10, 11	UGT2B7_8_10_11	0.29	0.41	0.91
NM_000963	Cyclooxygenase 2	COX2	0.32	0.45	0.98
NM_030753	Wingless-type MMTV integration site family, member 3	WNT3	0.33	0.32	0.34
NM_001880	Activating transcription factor 2	ATF2	3.08	1.88	0.41
DHA 24 h					
NM_000778	Cytochrome P450, family 4, subfamily A, polypeptide 11	CYP4A11	0.07	0.10	0.12
NM_000848	Glutathione S-transferase mu 2	GSTM2	0.12	0.11	0.11
NG_002601	UDP glucuronosyltransferase 1 family, polypeptide A3	UGT1A3_4_5	2.24	0.60	0.09
NM_004324	BCL2-associated X protein	BAX_2	2.25	2.26	0.53
NM_000146	Ferritin	FTL	2.43	0.84	0.04
NM_000765	Cytochrome P450, family 3, subfamily A, polypeptide 7	CYP3A7	3.02	3.20	0.43
NM_014465	Sulfotransferase family 1B, member 1	SULT1B1	3.02	3.60	0.52

Data are presented as fold change (FC) compared to the respective ethanol control for each experiment. A FC ≥ 2 was defined as gene up-regulation whereas a FC ≤ 0.33 was used as cut-off for down-regulated genes. Two-tailed t test was performed using Excel software

concentration (50 μM) of EPA ($n = 3$), DHA ($n = 2$) or alternatively 0.1% ethanol. The stock solution of each non-esterified fatty acid was purchased and dissolved in ethanol, thus ethanol was chosen to be the appropriate control. RNA was extracted after 10 and 24 h of incubation with the RNeasy Mini PLUS Kit (Qiagen GmbH, Hilden, Germany). RNA was quantified with the Nanodrop (Peqlab, Erlangen, Germany) and RNA integrity number (RIN) measured by Bioanalyzer (Agilent Technologies Deutschland GmbH, Böblingen, Germany). A control RNA which was labelled with Cy3 for each array was produced using untreated HT29 human colon carcinoma cells. Equal amounts of RNA (1 μg, RIN 8.5–10) were used for synthesising first strand of cDNA by Superscript II reverse transcriptase. A second strand cDNA was synthesized afterwards using Polymerase I, RNaseH and *E.coli* DNA ligase (Invitrogen GmbH, Karlsruhe, Germany). After clean-up of the product the double stranded cDNA was used for amplifying cRNA in order to increase the amount of RNA used for cDNA labelling with Cy3- and Cy5-dCTPs (GE Healthcare, Braunschweig, Germany). The reaction product was purified to yield clean, labelled and single

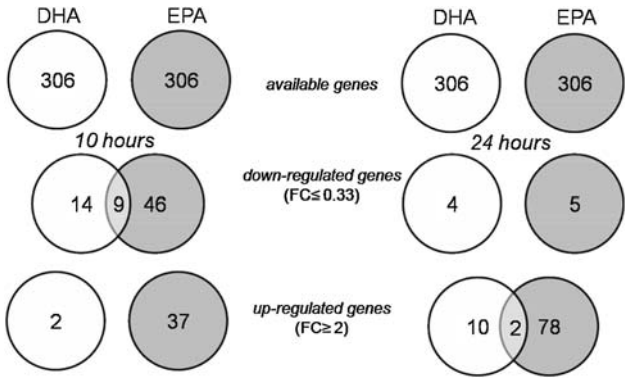

Fig. 1 Venn diagram for cDNA results comparing effects of DHA (*white circles*) and EPA (*grey circles*) in LT97 after 10 (*left panel*) or 24 h (*right panel*) of treatment. The number of genes are shown, which are down- or up-regulated for indicated treatments, overlapping numbers are the result of genes which are regulated by both PUFA in the same manner

stranded cDNA which was hybridised with a customised cDNA array (PIQOR, Miltenyi Biotec, Bergisch-Gladbach, Germany) containing 300 genes of interest and 6 housekeeping genes as described previously (11). Prior to this, the glass slide arrays were preheated and prehybridised. The final hybridisation step was carried out overnight in a water bath (65°C) in a humidified hybridisation chamber. After this, the custom arrays were washed and dried prior to scanning (MArS array reader, Ditabis, Pforzheim, Germany). Fluorescence intensities were analysed using the GeneSpotter software (MicroDiscovery, Berlin, Germany). LOESS-corrected raw data were normalised first in relation to the reference sample and second by setting the value for the housekeeping gene GAPDH equal to one. Data are presented as fold change (FC) compared to the respective ethanol control for each experiment.

Results and discussion

Using a stringent cut-off criteria ($\leq$0.33, $\geq$2) for the obtained FC, 16 genes were differentially expressed in LT97 after treatment with DHA for 10 h (2 up, 14 down) and 14 (10 up, 4 down) after 24 h. Using the same criteria, treatment with EPA resulted in a higher number of differentially expressed genes after both treatment times: 83 (37 up, 46 down) genes were modulated after 10 h, 83 (78 up, 5 down) after 24 h. Examples of modulated genes are given in a summarised form in Table 1. Only a few genes were modulated by both DHA and EPA in the same manner: 9 of the genes were down-regulated by both PUFAs after 10 h and 2 of the genes were up-regulated after 24 h (see Fig. 1).

There was a marked influence of *n*-3 PUFA on the expression of several gene types (Table 1). Of these, some could result in functional effects such as altered biotransformation of phase I [e.g., up-regulation of *CYP3A7* and *FMO5* (24 h)] and phase II [up-regulation, e.g. *GSTT2* (10), *SULT1B1*, *CHST8*, *UGT1A* family, *UGT2B* family down-regulation (10 h)], cell cycle control (e.g. up-regulation of *cyclin dependent kinase 2*), modulation of signalling pathways [e.g. down-regulation of *Wnt3* (10 h), up-regulation of *NFkB* (24 h)], apoptosis [e.g., down-regulation of *casp8* (10 h), *bax* up-regulation (24 h)], and inflammation (e.g. down-regulation of *COX2*). The modulation of gene expression by the *n*-3 PUFAs EPA and DHA has to be verified using real time PCR.

Our data show that *n*-3 PUFAs from fish oils may have a chemoprotective effect by modulating gene expression, which may prevent the development of adenomas to tumours. Our novel approach of studying this specific profile of gene expression modulation in preneoplastic human cells provides a rationale and relevant approach to identify target genes and agents that can contribute to chemoprevention.

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Conflict of interest statement There are no authors' conflict of interest which must be stated here.

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