Published in final edited form as:

J Surg Res. 2011 January ; 165(1): 75–81. doi:10.1016/j.jss.2009.04.022.

A high omega-3 fatty acid diet mitigates murine pancreatic precancer development

Matthew J. Strouch, MD¹, Yongzeng Ding, MD¹, Mohammad R. Salabat, MD¹, Laleh G. Melstrom, MD¹, Kevin Adrian¹, Christopher Quinn¹, Carolyn Pelham¹, Sambasiva Rao, MD², Thomas E. Adrian, PhD³, David J. Bentrem, MD¹, and Paul J. Grippo, PhD¹

¹Department of Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL

²Department of Pathology, Feinberg School of Medicine, Northwestern University, Chicago, IL

³Department of Physiology, Faculty of Medicine and Health Sciences, United Arab Emirates University, Al Ain, UAE

Abstract

Background—Diets containing omega-3 fat have been associated with decreased tumor development in the colon, breast, and prostate. We assessed the effects of a diet rich in omega-3 (ω -3) fat on the development of pancreatic precancer in elastase (EL)-Kras transgenic mice and examined the effect of an ω -3 fatty acid on pancreatic cancer cells *in vitro*.

Materials and Methods—Two cohorts of EL-Kras mice were fed a high ω -3 fat diet (23% menhaden oil) for 8 and 11 months and compared to age-matched EL-Kras mice fed standard chow (5% fat). Pancreata from all mice were scored for incidence and frequency of precancerous lesions. Immunohistochemistry was performed for proliferating cell nuclear antigen (PCNA) to assess proliferative index in lesions of mice fed either a high ω -3 or standard diet. *In vitro*, the effect of the ω -3 fatty acid, docosahexaenoic acid (DHA), on two pancreatic cancer cell lines was assessed. Cancer cell proliferation was assessed with an MTT assay, cell cycle analysis was performed by flow cytometry and apoptosis was assessed with Annexin/PI staining.

Results—The incidence, frequency, and proliferative index of pancreatic precancer in EL-Kras mice was reduced in mice fed a high ω -3 fat diet compared to mice fed a standard chow. *In vitro*, DHA treatment resulted in a concentration dependent decrease in proliferation through both G1/G0 cell cycle arrest and induction of apoptosis.

Conclusions—A high ω -3 fat diet mitigates pancreatic precancer by inhibition of cellular proliferation through induction of cell cycle arrest and apoptosis.

Keywords

Pancreatic Cancer; Chemoprevention; Omega-3; Apoptosis

© 2009 Elsevier Inc. All rights reserved.

Corresponding Author: Paul Grippo, PhD. Robert H. Lurie Comprehensive Cancer Center Feinberg School of Medicine Northwestern University 303 E. Superior Street Chicago, IL 60611 USA Phone (312) 503–3490 Fax (312) 503–0386 Email: p-grippo@northwestern.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

The importance of early cancer detection has been demonstrated in breast, prostate, and colon cancer whereby early treatment has resulted in improved survival (1-3). While there are currently no ideal diagnostic modalities to detect early pancreatic cancer, imaging techniques are evolving that have greater sensitivity to detect dysplastic changes in the pancreas (4). Therefore, identifying chemopreventive measures that could prevent the process of malignant transformation are needed. One promising area of chemopreventive investigation is the role of dietary fat in the development of cancer(5). *In vivo* studies in a hamster and rat models of chemically induced pancreatic cancer have shown a protective effect of dietary fish oil (ω -3 fat) supplementation(6,7). Although there is some degree of protection from cancer growth in animals supplemented with fish oil, there is no effective tumor regression or improvement in survival demonstrated in humans with advanced pancreatic cancer receiving ω -3 supplementation (8). Nonetheless, ω -3 supplementation may still have a preventive effect without having a chemotherapeutic effect, although this has never been studied prospectively in humans or mice.

In order to examine the role of fatty acids as a chemopreventive agent, we employed a mouse model that recapitulates pancreatic precancer. In humans, the most prominent genetic event observed in both Pancreatic Intraepithelial Neoplasias (PanINs) and pancreatic cancer is a mutation in the Kras allele, which causes constitutive signaling through the ras cascade. We have previously reported the development of a transgenic mouse model which targets human mutant Kras gene expression to the pancreas using a rat elastase promoter (9). Within the pancreas of these mice, precancerous lesions develop which are histologically similar to human PanINs, thus representing an ideal *in vivo* model to evaluate the effect of dietary changes on precancer development. These lesions can progress to invasive disease upon loss of TSG (p16) or other gene product such as Pigment Epithelium-Derived Factor (PEDF) (10).

While some studies have evaluated the effects of different fatty acid diets on rodents with chemically induced pancreatic cancer, there have been no *in vivo* studies examining the effects of high ω -3 diets on mice with genetic mutations that predispose them to pancreatic precancer. In this study, we evaluated the effects of high ω -3 fatty acid- supplemented diets on mice predisposed to the development of pancreatic precancer and examined the mechanisms *in vitro* through which alterations in lesion development may occur.

Materials and Methods

Production of transgenic study groups

EL-Kras transgenic mice were previously generated by microinjection of FVB/N fertilized mouse embryos and subsequent implantation into pseudopregnant females as described in a prior publication and transgenic mice developed pancreatic precancer at roughly a 50% penetrance at 12 months of age (9). PCR genotyping of EL-Kras mice has demonstrated that all males were positive for the transgene while all females were negative. EL-Kras mice were subjected to tail biopsy and PCR evaluation of genomic DNA from mouse tail digests (see below). EL-Kras male mice were also bred to C57BL/6 (B6) female mice to generate EL-Kras FVB6 F1 mice (EL-Kras F1) which developed roughly a 100% penetrance of pancreatic precancer by 6 months of age. EL-Kras F1 mice were used for this diet study.

Tail biopsy and PCR genotyping

A piece of mouse tail was digested with proteinase K (Fermentas) solution in GNT Buffer (50mM KCl, 1.5mM MgCl₂, 10mM Tris-HCl, 0.01% gelatin, 0.45% NP40, and 0.45% Tween-20 pH=8.5). The digested, heat-inactivated supernatant was used as a DNA template

in the following 25 μ l PCR reactions: 1 μ M of each primer (EL-Kras 1f primer 5' 3' and 2r primer 5' 3') in a solution with 1 $\times$ PCR Buffer, 1.5mM MgCl₂, 1 μ M dNTPs, and 0.2 – 0.4 U Taq polymerase (Fermentas or Promega) was used in a standard PCR reaction. The 25 μ l samples were incubated at 94°C for 3–5 minutes followed by 35 cycles of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute followed by 5 minutes at 72°C and an indefinite soak at 4–10°C.

Dietary Studies & Histology

Groups of mice were administered *ad libitum* either a standard chow (~5% fat) or a diet with 23% menhaden oil (ω -3). The high omega 3 fat diet has a 1:2.5 omega-3:omega-6 polyunsaturated fatty acid ratio while the standard diet has a 1:9.6 omega-3:omega-6 polyunsaturated fatty acid ratio (For further diet details see Table 1). Five EL-Kras F1 mice were fed a 23% menhaden oil diet and sacrificed at 8 months of age while six EL-Kras F1 mice were fed 23% menhaden oil and sacrificed at 11 months of age. Mice on the high ω -3 diet were compared to an equal number of age-matched EL-Kras F1 mice fed the standard chow. Following euthanasia, mouse pancreatic tissue was fixed in 10% buffered formalin overnight. Tissue blocks were sectioned at 4–6 μ m and placed on positively charged slides. Following removal of paraffin by xylenes and subsequent hydration, the tissue was stained with H&E and then dehydrated to xylenes and mounted with a cover slip. H&E stained tissue was scored for the presence of ductal carcinoma in situ (pancreatic precancer). Several parameters were noted including incidence (number of mice with lesions per number of mice with genotype) and frequency (number of lesions per random section/per mouse).

Immunohistochemistry

After deparaffinization, the slides were heated in the microwave in Dako antigen retrieval solution [(S1699), Dako (Carpinteria, CA)] and then washed in Dako wash buffer (S3006). Sections were blocked with 0.5% BSA in PBS for 30 minutes at room temperature followed by Dako peroxidase block for 30 minutes. Primary anti-PCNA (Proliferating Cell Nuclear Antigen) antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was placed on sections at a concentration of 1:1000 at room temperature for 1 hour. Sections were then treated with Dako secondary antibody (anti-mouse IgG) HRP-linked (Dako Envision Kit K4008) for 30 minutes at room temperature and developed with DAB reagent for 30 sec. Counterstaining was performed with Gills-2 hematoxylin. Immunohistochemistry for PCNA was independently evaluated manually by two investigators (P.G. and M.B.), verified by ImageJ software (National Institute of Health, Bethesda MD.), and calculated as percentage of positive nuclei per lesion.

Cell Proliferation Assay

Human pancreatic cancer cell lines BxPC-3 and AsPC-1 were obtained from the American Type Culture Collection and cultured in DMEM supplemented with 10% fetal bovine serum in a 37°C incubator in a humidified atmosphere of 5% CO₂. Fatty acid-free bovine serum albumin (BSA, sigma Chemical) was prepared as a 125 μ mol/L solution in DMEM. A purified ω -3 fat, Docosahexaenoic acid (DHA), purchased as a sodium salt (Sigma Chemical), was solubilized to 600 μ mol/L stocks in the BSA medium. BSA was used as a carrier protein for DHA and maintained at a constant ratio of 4.8:1 for all experiments based on previously published *in vitro* ω -3 culture models(11). Cell growth was measured using the 3-(4,5 dimethylthiazol-2yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma) colorimetric dye reduction method. Pancreatic cancer cells were seeded at a density of 1×10^4 cells for both ASPC-1 and BxPC-3 per well in 96-well plates in complete cell culture medium containing 0.5% FBS. After 24 hours, culture medium was then replaced with serum-free medium together with indicated reagents. After 48 hours, 10 μ L MTT with 5 mg/mL concentration was added to the medium and cultured for another 2 h. Then, the medium

was discarded and 200 μ L DMSO was added into each well. The plate was then put on a shaker for 10 min. The absorbencies of each well were read at a wavelength of 570 nm using a spectrophotometric plate reader (Bio-Tek Instruments). Proliferation assays were repeated in triplicate for each cell line.

Analysis of cellular DNA content by flow cytometry

AsPC-1 and BxPC-3 cells were grown to 50% confluence in T25-cm² flasks and then incubated in Dubelco's Modified Eagles Medium (DMEM) with 0.5% FBS overnight. Cells were treated with either DHA (40 μ M) or BSA (40 μ M) for 48 hours and then harvested with a trypsin-EDTA solution to produce a single-cell suspension and were then washed twice with PBS. The cells were suspended in 0.5mL PBS, fixed in 70% ethanol at 4°C, centrifuged at $\times 300g$ for 10 minutes and then washed with PBS. After resuspension in 1ml PBS, the cells were incubated with 5 μ L Rnase I (10mg/ml) at 37°C for half an hour. Then, 150 μ L of propidium iodide (400g/mL, Sigma (St. Louis, MO) was added and the cells were shaken for 2 hours in the dark at room temperature. The samples were analyzed by flow cytometry.

Assay for apoptosis

AsPC-1 and BxPC-3 cells were grown to 50% confluence in T25-cm² flasks and then incubated in serum DMEM with 0.5% FBS overnight. Cells were treated with either DHA (40 μ M) or BSA (40 μ M) for 48 hours and then harvested with a trypsin-EDTA solution to produce a single-cell suspension and were then washed twice with PBS. Annexin V-FITC and PI analysis (BD Biosciences kit 556547) was performed according to manufacture protocol (BD Biosciences, San Jose, CA) and samples were analyzed by flow cytometry. Cell treatment with DHA and Annexin-PI staining was repeated in triplicate for each cell line.

Statistical Analysis

Data were analyzed by ANOVA, followed by Dunnett's Multiple Comparison Test as appropriate for post-hoc testing and paired t-tests where appropriate. The analyses were performed with the Prism software package (Graphpad, San Diego, CA). Data were expressed as mean $\pm$ SEM.

Results

Omega-3 fatty acids mitigate pancreatic precancer *in vivo*

In order to assess the phenotypic effect of the ω -3 fat diet on pancreatic carcinogenesis, five ELKras F1 transgenic mice were fed a high ω -3 fat diet and sacrificed at eight months while six EL-Kras F1 mice were fed a high ω -3 fat diet and sacrificed at eleven months. Each cohort of mice was compared to an equal number of age matched EL-Kras F1 mice fed a standard chow. On histological examination, all of the EL-Kras F1 mice on a standard diet developed precancerous pancreatic lesions (representative lesion shown in Figure 1, panel A) while only 3 of 5 eight-month and 3 of 6 eleven-month old mice on the ω -3 fat diet demonstrated precancer on histological examination. (Figure 1, panels C and D) The histology of the precancerous lesions in mice fed the high omega-3 fat diet was identical to that of mice fed the standard diet, however, there was a tendency towards smaller lesions in mice fed the high omega-3 diet. The frequency of precancerous lesions in eight month and eleven month old ω -3 diet mice were 1.0 $\pm$ 0.55 and 2.2 $\pm$ 1.28 lesions per mouse respectively while the eight and eleven month old standard diet mice had a frequency of 4.8 $\pm$ 0.37 and 9.5 $\pm$ 2.25 lesions per mouse respectively. [(p<0.0001 and p<0.01 for the 8 month and 11 month groups respectively) Figure 1, panels E and F]. There was no

statistically significant difference in weights between ω -3 fed mice compared to age-matched mice on standard chow (Data not shown).

Omega-3 fatty acid diet inhibits precancer proliferation *in vivo*

Due to evidence that high fat diets may affect the rate of pancreatic cancer proliferation *in vitro* (12-15), we performed immunohistochemistry for PCNA, a nuclear marker expressed by proliferating cells, on the precancerous lesions to assess whether the diets affect pancreatic precancer proliferation rates *in vivo*. All of the EL-Kras F1 mice with precancer on either a standard or high ω -3 fat diet were immunohistochemically stained with PCNA and the lesions were scored for percentage of positive nuclei per lesion for each mouse examined. (Representative IHC demonstrated in Figure 2, panels A and B) In mice fed the high ω -3 fat diet, 7.0% of precancer cells stained positive for PCNA compared to 24.3% in age matched mice fed the standard diet. [(p<0.05) Figure 2 Panel C]

Omega-3 fatty acid inhibits pancreatic cancer proliferation *in vitro*

In order to implicate the ω -3 fat as the causative agent for the alterations in pancreatic precancer demonstrated *in vivo*, we evaluated the effect of an ω -3 fat, DHA, on pancreatic cancer cells *in vitro*. In both BxPC-3 and AsPC-1 pancreatic cancer cells, we demonstrated a concentration dependent decrease in cellular proliferation in cancer cells treated with DHA compared to untreated cells at 48 hours. (Figure 3, panels A and B) In both cell lines, a statistically significant decrease in proliferation was obtained at a concentration of 40 μ m DHA and above (p<0.05) and thus a concentration of 40 μ m DHA was used for the following *in vitro* experiments.

Omega-3 fatty acid induces G1/G0 Cell cycle arrest and apoptosis *in vitro*

After demonstrating a decrease in proliferative rate in pancreatic cancer cells treated with ω -3 fat, we sought to further elucidate the mechanism of this decrease by performing cell cycle analysis and flow cytometry for apoptosis in cells treated with DHA. In both AsPC-1 and BxPC-3 cells, we demonstrated an increase in G1 arrest in cells treated with 40 μ m DHA compared to controls. [(78.5% vs. 69.5% and 89.8% vs 84.4% respectively) Figure 4, panels A and B] We next performed flow cytometry with Annexin and PI staining to assess apoptosis rates in AsPC-1 and BxPC-3 cells treated with 40 μ m DHA compared to controls. In both AsPC-1 and BxPC-3 cells, there was a statistically significant induction in apoptosis following a 48 hour treatment with DHA. [(17.6% vs. 9.7% and 30.2% vs. 21.6%, p<0.05 and p<0.01 respectively) Figure 5, panels A and B]

Discussion

There is increasing evidence that dietary intake of ω -3 fatty acids suppress progression of breast, prostate and colon cancers. (16-19) However, there is limited understanding of how dietary fatty acids may affect the process of pancreatic carcinogenesis and cancer progression. Decreased tumor growth rates in BOP-induced pancreatic cancer were observed in hamsters fed a high ω -3 fat diet. (20) To date, all of the *in vivo* studies regarding the effect of different polyunsaturated fatty acid (PUFA) diets during pancreatic carcinogenesis have utilized carcinogen-induced (7,20-23) and xenograft (24,25) models that develop invasive pancreatic cancer. The goal of this study was to assess the possible chemopreventive role of a diet rich in ω -3 fat during the development of precancer *in vivo* and determine if this chemopreventive effect was generated by a similar mechanism to that observed in pancreatic cancer cell lines following treatment with an ω -3 fatty acid. To our knowledge, this is the first study to evaluate the effects of an ω -3 fat diet on transgenic mice predisposed to the development of pancreatic precancer.

The most striking finding in this study was the significantly decreased incidence and frequency of pancreatic precancer in EL-Kras F1 mice fed a high ω -3 fat diet versus those fed a standard diet. This change occurred early in the eight-month old mice and persisted in the eleven-month old mice. In addition, the cellular proliferation rate in precancerous lesions was significantly reduced in the ω -3 fatty acid group compared to the standard diet group. Since the Kras mutation is targeted to the acinar compartment, the ω -3 diet likely plays a role in both retarding the transformation process, which leads to the initial formation of pancreatic precancer and then dampening the proliferative ability of the precancerous lesions once they develop. Previous findings in prostate (26), mammary (27), skin (28), and colon(29) in a precancerous setting demonstrated that administration of high ω -3 diets generated an environment that suppressed tumor establishment. Our results extend this idea into pancreatic precancer development and support previously identified *in vivo* effects of high fat diets during pancreatic carcinogenesis but were generated in a precancerous setting in the pancreas. The suppressive nature of an ω -3 fat diet on EL-Kras mice was even more pronounced than those observed in BOP-induced pancreatic cancer in hamsters(20) since some EL-Kras F1 ω -3 fed mice do not develop precancer.

Two issues remain regarding the phenotypic effects of high PUFA diets on the development of pancreatic precancer *in vivo*: (1) other potential variables that may contribute to these changes and (2) the mechanisms responsible for cellular/tissue abnormalities leading to precancer. When examining the differences between the high omega-3 and standard diets (see Table 1), the ω -3 fat diet had a higher calorie content per gram than the standard diet. The only means to assess lower fat composition in an isocaloric setting would be to compensate calories from fat with calories from sugar, which changes the whole dynamic of this study from an evaluation of a high dietary omega-3 fat to the source of caloric intake. Even in a proposed isocaloric setting, total calorie intake would be difficult to maintain unless each mouse consumes the same amount of isocaloric diet. Nevertheless, EL-Kras F1 mice fed a high ω -3 fat diet had no significant difference in body weights compared to mice fed a control diet. Moreover, our *in vitro* data demonstrating a lower proliferation rate in pancreatic cancer cells treated with DHA supports our *in vivo* findings and implicates the ω -3 fat as the main factor associated the proliferative changes and precancer inhibition. Furthermore, we demonstrated that DHA affects proliferation through both induction of G1 arrest and apoptosis.

In conclusion, we demonstrated for the first time, that a diet high in ω -3 fat may mitigate the development of pancreatic precancer. Omega-3 fats appear to have this effect by inhibiting pancreatic lesion proliferation through induction of G1 cell cycle arrest and apoptosis. More work is needed to further elucidate the mechanisms of action of ω -3 fatty acids and is the focus of our next study. The present study serves as provocative preclinical data that dietary intake of omega-3 fat may mitigate the development of pancreatic precancer or cancer in high-risk pancreatic cancer kindreds. Likely, the most effective method to alter a patient's intake of omega-3 fat would be in the form of vitamin supplementation (fish oil, flax seed oil etc.). Ultimately, a high ω -3 fat diet may hold promise as a means of impeding the development and progression of pancreatic precancer.

Acknowledgments

We gratefully acknowledge Dr. Diane Birt for her assistance with diet formulations. We appreciate the assistance of Meg Bowden for helping procure non-transgenic control tissue. We kindly acknowledge the consultation of Dr. Eric Sandgren regarding background strain issues with EL-Kras transgenic mice.

Financial Support: This study was funded in part by a National Institute of Health (N.I.H.) R21 (P.G. 5-R21-CA123041-02).

References

1. Lamb DS, Slaney D, Smart R, Nacey JN, Russell G, Scott B, Johnson CA, Adams JD, Moran S, Delahunt B. Prostate cancer: the new evidence base for diagnosis and treatment. *Pathology* 2007;39:537–544. [PubMed: 18027255]
2. Levin B, Lieberman DA, McFarland B, Andrews KS, Brooks D, Bond J, Dash C, Giardiello FM, Glick S, Johnson D, Johnson CD, Levin TR, Pickhardt PJ, Rex DK, Smith RA, Thorson A, Winawer SJ. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *Gastroenterology* 2008;134:1570–1595. [PubMed: 18384785]
3. Perry N, Broeders M, de Wolf C, Tornberg S, Holland R, von Karsa L. European guidelines for quality assurance in breast cancer screening and diagnosis. Fourth edition--summary document. *Ann Oncol* 2008;19:614–622. [PubMed: 18024988]
4. Edelman RR. MR Imaging of the Pancreas: 1.5T versus 3T. *Magn Reson Imaging Clin N Am* 2007;15:349–353. [PubMed: 17893054]
5. Berquin IM, Edwards IJ, Chen YQ. Multi-targeted therapy of cancer by omega-3 fatty acids. *Cancer Lett* 2008;269:363–377. [PubMed: 18479809]
6. Gregor JI, Heukamp I, Kilian M, Kiewert C, Schimke I, Kristiansen G, Walz MK, Jacobi CA, Wenger FA. Does enteral nutrition of dietary polyunsaturated fatty acids promote oxidative stress and tumour growth in ductal pancreatic cancer? Experimental trial in Syrian Hamster. *Prostaglandins Leukot Essent Fatty Acids* 2006;74:67–74. [PubMed: 16226437]
7. O'Connor TP, Roebuck BD, Peterson FJ, Lokesh B, Kinsella JE, Campbell TC. Effect of dietary omega-3 and omega-6 fatty acids on development of azaserine-induced preneoplastic lesions in rat pancreas. *J Natl Cancer Inst* 1989;81:858–863. [PubMed: 2724351]
8. Jho DH, Cole SM, Lee EM, Espat NJ. Role of omega-3 fatty acid supplementation in inflammation and malignancy. *Integr Cancer Ther* 2004;3:98–111. [PubMed: 15165497]
9. Grippo PJ, Nowlin PS, Demeure MJ, Longnecker DS, Sandgren EP. Preinvasive pancreatic neoplasia of ductal phenotype induced by acinar cell targeting of mutant Kras in transgenic mice. *Cancer Res* 2003;63:2016–2019. [PubMed: 12727811]
10. Grippo P, R. T, Pelham C, Stellmach V, Crawford SE, Rao SM, Ding XZ, Talamonti MS, Bell RH, Adrian TE. Loss of pigment epithelium-derived factor (PEDF) expression enhances pancreatic intraepithelial neoplasia (PanIN) frequency and pancreatic fibrosis in vivo. *Pancreas* 2005;31:443.
11. Sun H, Berquin IM, Edwards IJ. Omega-3 polyunsaturated fatty acids regulate syndecan-1 expression in human breast cancer cells. *Cancer Res* 2005;65:4442–4447. [PubMed: 15899837]
12. Dekoj T, Lee S, Desai S, Trevino J, Babcock TA, Helton WS, Espat NJ. G2/M cell-cycle arrest and apoptosis by n-3 fatty acids in a pancreatic cancer model. *J Surg Res* 2007;139:106–112. [PubMed: 17291531]
13. Hering J, Garrean S, Dekoj TR, Razzak A, Saied A, Trevino J, Babcock TA, Espat NJ. Inhibition of proliferation by omega-3 fatty acids in chemoresistant pancreatic cancer cells. *Ann Surg Oncol* 2007;14:3620–3628. [PubMed: 17896154]
14. Merendino N, Molinari R, Loppi B, Pessina G, M DA, Tomassi G, Velottia F. Induction of apoptosis in human pancreatic cancer cells by docosahexaenoic acid. *Ann N Y Acad Sci* 2003;1010:361–364. [PubMed: 15033753]
15. Shirota T, Haji S, Yamasaki M, Iwasaki T, Hidaka T, Takeyama Y, Shiozaki H, Ohyanagi H. Apoptosis in human pancreatic cancer cells induced by eicosapentaenoic acid. *Nutrition* 2005;21:1010–1017. [PubMed: 16157238]
16. Boudreau MD, Sohn KH, Rhee SH, Lee SW, Hunt JD, Hwang DH. Suppression of tumor cell growth both in nude mice and in culture by n-3 polyunsaturated fatty acids: mediation through cyclooxygenase-independent pathways. *Cancer Res* 2001;61:1386–1391. [PubMed: 11245439]
17. Rose DP. Effects of dietary fatty acids on breast and prostate cancers: evidence from in vitro experiments and animal studies. *Am J Clin Nutr* 1997;66:1513S–1522S. [PubMed: 9394709]
18. Rose DP, Connolly JM. Omega-3 fatty acids as cancer chemopreventive agents. *Pharmacol Ther* 1999;83:217–244. [PubMed: 10576293]

19. Rose DP, Connolly JM. Dietary fat and breast cancer metastasis by human tumor xenografts. *Breast Cancer Res Treat* 1997;46:225–237. [PubMed: 9478277]
20. Birt DF, Julius AD, White LT, Pour PM. Enhancement of pancreatic carcinogenesis in hamsters fed a high-fat diet ad libitum and at a controlled calorie intake. *Cancer Res* 1989;49:5848–5851. [PubMed: 2790796]
21. Appel MJ, van Garderen-Hoetmer A, Woutersen RA. Effects of dietary linoleic acid on pancreatic carcinogenesis in rats and hamsters. *Cancer Res* 1994;54:2113–2120. [PubMed: 8174115]
22. Cave WT Jr. Dietary n-3 (omega-3) polyunsaturated fatty acid effects on animal tumorigenesis. *Faseb J* 1991;5:2160–2166. [PubMed: 1673664]
23. Herrington MK, Permert J, Kazakoff KR, Pour PM, Zuker KA, Bilchik AJ, Adrian TE. Effects of high fat diet and cholecystokinin receptor blockade on pancreatic growth and tumor initiation in the hamster. *Carcinogenesis* 1993;14:1021–1026. [PubMed: 7684953]
24. Smith JP, Kramer S, Bagheri S. Effects of a high-fat diet and L364,718 on growth of human pancreas cancer. *Dig Dis Sci* 1990;35:726–732. [PubMed: 2344806]
25. Swamy MV, Citineni B, Patlolla JM, Mohammed A, Zhang Y, Rao CV. Prevention and treatment of pancreatic cancer by curcumin in combination with omega-3 fatty acids. *Nutr Cancer* 2008;60(Suppl 1):81–89. [PubMed: 19003584]
26. Berquin IM, Min Y, Wu R, Wu J, Perry D, Cline JM, Thomas MJ, Thornburg T, Kulik G, Smith A, Edwards IJ, D'Agostino R, Zhang H, Wu H, Kang JX, Chen YQ. Modulation of prostate cancer genetic risk by omega-3 and omega-6 fatty acids. *J Clin Invest* 2007;117:1866–1875. [PubMed: 17607361]
27. Luijten M, Verhoef A, Dormans JA, Beems RB, Cremers HW, Nagelkerke NJ, Adlercreutz H, Penalvo JL, Piersma AH. Modulation of mammary tumor development in Tg.NK (MMTV/c-neu) mice by dietary fatty acids and life stage-specific exposure to phytoestrogens. *Reprod Toxicol* 2007;23:407–413. [PubMed: 17229545]
28. Black HS, Rhodes LE. The potential of omega-3 fatty acids in the prevention of non-melanoma skin cancer. *Cancer Detect Prev* 2006;30:224–232. [PubMed: 16872755]
29. Kimura Y, Kono S, Toyomura K, Nagano J, Mizoue T, Moore MA, Mibu R, Tanaka M, Kakeji Y, Maehara Y, Okamura T, Ikejiri K, Futami K, Yasunami Y, Maekawa T, Takenaka K, Ichimiya H, Imaizumi N. Meat, fish and fat intake in relation to subsite-specific risk of colorectal cancer: The Fukuoka Colorectal Cancer Study. *Cancer Sci* 2007;98:590–597. [PubMed: 17425596]

Figure 1.

Representative H&E sections of pancreatic precancer lesions in (a) a mouse on standard diet at 100× magnification and (b) a mouse on the high omega-3 fat diet at 200× magnification. The incidence of pancreatic precancer was (c) 60% in ω-3 fed mice at 8 months of age and (d) 50% in ω-3 fed mice at 11 months of age. There was a 100% incidence of precancer in standard diet mice at both 8 and 11 months. The frequency of pancreatic precancer was significantly less in mice fed the ω-3 diet compared to control mice at (e) 8 months and (f) 11 months. ($p<0.0001$ and $p<0.01$ respectively)

Figure 2.

Representative IHC for proliferating nuclear cell antigen (PCNA) in pancreatic precancer lesions of (a) a standard diet fed EL-Kras mouse and (b) an ω-3 diet fed EL-Kras mouse. (c) Lesions from mice fed the high ω-3 diet demonstrated a significantly less proliferative rate than those in mice fed the standard diet. [(7.0 ± 0.6% vs. 24.3 ± 2.4%) p<0.05]

Figure 3.

Concentration response curve for DHA treatment. C represents control cells while T represents DHA treated cells. Concentrations are in μM . We demonstrated a concentration dependent decrease in (a) BxPC-3 and (b) AsPC-1 pancreatic cancer cell proliferation compared to control cells by MTT assay when cells were treated with DHA for 48 hours. A statistically significant decrease in proliferation was obtained in both cell lines at a concentration of $40\mu\text{M}$ DHA and higher.

Figure 4. Flow cytometry for cell cycle. In both (a) BxPC-3 and (b) AsPC-1 pancreatic cancer cells, there was an induction in G1 growth arrest when cells were treated with 40 μ M DHA for 48 hours.

Figure 5.

In vitro assay for apoptosis. Both AsPC-1 and BxPC-3 pancreatic cancer cells were treated with 40 μ M DHA for 48 hours and annexin-PI flow cytometry was performed to assess the percentage of cells undergoing apoptosis. There was a statistically significant increase in the number of cells undergoing apoptosis in both (a) BxPC-3 and (b) AsPC-1 pancreatic cancer cell lines. (17.6% vs. 9.7% and 30.2% vs. 21.6%, $p < 0.05$ and $p < 0.01$ respectively)

Table 1**Diet Composition**

Ingredient	Standard Diet (g/kg)	High Omega-3 Diet (g/kg)
Corn Oil	0	0
Total Omega-6 Fatty Acids	27.9	123
Fish Oil	0	230
Total Omega-3 Fatty Acids	2.9	49.9
Omega-3:Omega-6 Fatty acid ratio	1:9.6	1:2.5
Calculated Values		
Protein	19%	18%
Fat	5.60%	23%
Fiber	4.5% [§]	5%
Ash	6.50%	2.80%
Moisture	10–12%	<5%
Carbohydrate	40.30%	46.20%
Protein Energy	0.76 kcal/g	0.72 kcal/g
Fat Energy	0.51 kcal/g	2.07 kcal/g
Carbohydrate Energy	1.61 kcal/g	1.85 kcal/g
Total Energy	2.90 kcal/g	4.64 kcal/g

[§] = value represents crude fiber while actual fiber may be closer to 14–16%