Conclusions: These data suggest that PL suppresses tumour growth, invasion, and angiogenesis through the inhibition of Wnt/β-catenin signalling in certain colon cancer cells.
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1032 POSTER

Docosahexaenoic Acid Inhibits Cell Growth Through PTEN/PI3K/Akt Signaling Pathway in A549 Human Non-small Cell Lung Carcinoma Cells

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Background: Lung cancer is leading cause of all cancer deaths. Although omega-3 polyunsaturated fatty acids (ω3-PUFAs) have been reported to inhibit cell growth in several cancers, the anti-cancer mechanism of ω3-PUFAs on lung cancer is still unclear. In this study, we have investigated the mechanism of anti-cancer action of docosahexaenoic acid (DHA), one of ω3-PUFAs, in A549 human non-small cell lung cancer (NSCLC) cell line.
Material and Methods: Cell viability was analyzed using the MTT assay. Signaling proteins were detected by Western blot assay. TUNEL assay and FACS analysis were used for measuring apoptotic cell death. Lipofectamin was used to transfect Akt gene to cells.
Results: Following treatment of DHA, the viability of A549 cells was decreased in a dose-dependent manner. DHA induced apoptotic cell death as revealed by increased cleaved PARP, TUNEL positive cells and subG1 population. The amounts of PI3K and phospho-Akt proteins were decreased after DHA treatment in dose-dependent manner. In addition, DHA decreased the level of phospho-phosphatase and tensin homolog deleted on chromosome ten (p-PTEN) protein, which is an inactive form of PTEN. Moreover, transient transfection of full length of Akt cDNA into A549 cells partially restored DHA-dependent inhibition of cell growth compared with the cells transfected with kinase dead form of Akt. Taken together, these data suggest that inhibition of PI3K/Akt signaling pathway may be related to anti-cancer action of DHA in A549 human NSCLC cells.
Conclusions: Docosahexaenoic acid-induced cytoxicity may be related to PTEN/PI3K/Akt signaling pathway in A549 human non-small cell lung carcinoma cells. Utilization of DHA may represent a potential effective therapy for the chemoprevention and treatment of human non-small cell lung cancer.
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1033 POSTER

Fat-1 Gene Expression Inhibits Human Cervical Cancer Cells Growth in Vitro and in Vivo

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Background: Omega 3-polyunsaturated fatty acids (ω3-PUFAs) are known to inhibit proliferation of cancer cells; in contrast, ω6-PUFAs promote the growth of cancer cells. The fat-1 gene of the Caenorhabditis elegans encodes a ω3-desaturase that catalyzes the conversion ω6-PUFAs to ω3-PUFAs and then increases the amount of ω3-PUFAs. Therefore, a stable cell line of fat-1 gene is useful to study the anti-cancer effects of ω3-PUFAs.
Material and Methods: Fat-1 gene stable cell lines (f-HeLa and f-SiHa) were established from HeLa and SiHa cervical cancer cells by transfection and antibiotic selection. The effects of fat-1 gene on cell proliferation and cell cycle were examined by MTT assay and FACS. Transwell migration assay was employed to analyze the migration ability of fat-1 stably expressed cells in vitro and the in vivo effect of fat-1 gene was evaluated in an athymic nude mouse f-HeLa tumour engraft model.
Results: The fat-1 gene expression significantly inhibited cervical cancer cell proliferation and f-HeLa cells showed an increase in the proportion of cells in G2/M phase comparing with the cells expressing the control vector (c-HeLa). In addition, when treating HeLa cells with a ω3-PUFA, DHA (docosahexaenoic acid), an enhanced proportion of cells in the G2/M phase was also observed, indicating that fat-1 gene inhibited cervical cancer cell proliferation by inducing a G2/M phase cell-cycle arrest. Furthermore, transwell migration assay for invasion indicated a reduction of cell migration in the f-HeLa cells when compared with that in the c-HeLa cells. Finally, the growth of f-HeLa cells in vivo was significantly reduced comparing with the c-HeLa cells when inoculated into nude mice.
Conclusions: Our results suggest that the expression of fat-1 gene prevents cervical tumour growth and indicate a cancer therapeutic approach of the ω3-PUFAs.
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1034 POSTER

Docosahexaenoic Acid Induces Autophagy Through p53/AMPK/mTor Signaling in Human Cancer Cells Harboring Wild-type p53

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Background: Although omega 3-polyunsatuarated fatty acids (omega 3-PUFA) induce cytotoxicity in several cancer cell lines, the exact mechanisms are not identified yet. In this study, we showed that autophagy, characterized by the sequestration of cytoplasmic material within autophagosomes for bulk degradation by lysosomes, is involved in the omega 3-PUFAs-induced cytotoxicity in wild-type p53 harbored human cancer cells.
Material and Methods: Autophagy was detected after docosahexaenoic acid (DHA), an omega 3-PUFA, exposure as indicated by induction of LC3 expression, and formation of autophagic vacuolization. Pifithrin-α, a p53 inhibitor and microRNA-p53 were employed to downregulate p53 activity and investigate the p53-involved autophagic activation in cancer cells treated with DHA.
Results: We found that DHA induced not only apoptosis but also autophagy in cancer cells harboring wild-type p53. DHA-induced autophagy was accompanied by loss of p53 and inhibition of p53 significantly increased the DHA-induced autophagy, suggesting that the DHA-induced autophagy is mediated by downregulation of p53. Further experiments showed that the mechanism of the DHA-induced autophagy associated with p53 attenuation, involved an increase in the active form of AMPK which attenuated the mTOR activity as revealed by p27 sequestration. In addition, compelling evidence for the interplay between autophagy and apoptotic cell death induced by DHA is supported by the findings that autophagy inhibition partially decreased the DHA-induced apoptotic cell death and further autophagy induction by p53 inhibitor enhanced apoptosis in response to treatment with DHA in cancer cells.
Conclusions: Our results demonstrate that autophagy is related to the DHA-induced cytotoxicity in wild-type p53 harbored cancer cells.
This work was supported by basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (2010–0016447 and 2010–0001290).

1035 POSTER

Genome-wide Promoter and CpG Island DNA Methylation Screening Identifies Novel Prognostic Markers and Distinct Pathway in Rectal Cancer

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Background: The prognostic utility of DNA methylation may be incorporated into an evolving strategy of organ preservation for rectal cancer. Current selection criteria for local therapy remain imprecise. A genome-wide approach could provide novel prognostic markers to guide decision-making and determine key pathways responsible in rectal cancer progression.
Methods: Methylcytidine antibody-bound DNA from 10 early, node-negative and 10 advanced, node-positive rectal cancers were immunoprecipitated and hybridised to 385K Nimblegen promoter array. Differential methylation signals were determined and analysed using the linear models for microarray data (Limma) method. Molecular functions and pathways were determined using the PANTHER classification system.
Results: Over 350 genes were differentially methylated (fold change >2, $P < 0.01$) between early and advanced cancers. A greater number of methylated genes were seen in advanced compared to early cancer, in the ratio of 2:1, suggesting a general accumulation of aberrant methylation during cancer progression. The majority of genes hypermethylated in advanced cancers were ion channels ($P = 1.23 \times 10^{-4}$) and transcription factors ($P = 2.71 \times 10^{-3}$). The Notch signaling pathway was over-presented with genes hypermethylated in advanced cancer ($P = 5.60 \times 10^{-3}$). The molecular function and pathway for genes showing greater methylation in early cancer was less clear.