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

K. Lim[1], N. Kim[1], K.S. Song[1], K. Jing[1], S. Jeong[1], S. Shin[1], H. Oh[1], J.I. Park[1], W.H. Yoon[1], B.D. Hwang[1]. *[1]Chungnam National University Medical School, Biochemistry, Daejeon, Korea*

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

K. Lim[1], K. Jing[1], K.S. Song[1], S. Shin[1], N. Kim[1], S. Jeong[1], H.D. Park[2], Y. Dai[3], W.H. Yoon[1], B.D. Hwang[1]. *[1]Chungnam National University Medical School, Biochemistry, Daejeon, [2]Dr. Park's Breast Clinic, Breast, Daejeon, Korea; [3]University of Pittsburg Medical School, Surgery, Pittsburgh, USA*

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

K. Lim[1], K. Jing[1], K.S. Song[1], S. Shin[1], N. Kim[1], S. Jeong[1], J.Y. Heo[1], H.D. Park[1], W.H. Yoon[1], B.D. Hwang[1]. *[1]Chungnam National University Medical School, Biochemistry, Daejeon, Korea*

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

K.J. Leong[1], W. Wei[1], L.A. Tannahill[1], G.M. Caldwell[1], J. James[1], C.E. Jones[1], D.G. Morton[1], S.P. Bach[1], G.M. Matthews[1]. *[1]University of Birmingham, School of Cancer Sciences, Birmingham, United Kingdom*

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.

Conclusion: This study has identified novel prognostic markers for rectal cancer. Validation by bisulphite pyrosequencing in an independent cohort is underway. Analysis of global methylation changes in rectal cancer, not previously reported, has provided an insight into key pathway that is responsible in disease progression and may be target for future therapeutic studies.

1036 POSTER

Thymoquinone-induced UHRF1 Ubiquitination is a Key Event for Challenging Apoptosis in Cancer Cells

M. Alhosin[1], A. Ibrahim[1], A. Boukhari[1], T. Sharif[1], J.P. Gies[1], C.D. Muller[2], V.B. Schini-Kerth[1], C. Bronner[1]. *[1]University of Strasbourg, CNRS UMR7213, Illkirch, [2]University of Strasbourg, CNRS UMR7200, Illkirch, France*

Background: UHRF1 (Ubiquitin-like containing PHD Ring Finger), an anti-apoptotic protein essential for cell proliferation, is over-expressed in several types of cancer. UHRF1 participates in a huge macro-molecular complex including DNMT1 (DNA methyltransferase 1), Tip60 (a histone acetyltransferase), HAUSP (a ubiquitin specific protease) and HDAC1 (a histone deacetylase). It has been shown that HAUSP protects, *in vitro*, UHRF1 from auto-ubiquitination and regulates its stability *in vivo*. We previously showed that thymoquinone (TQ), an anti-cancer drug, induced UHRF1 down-regulation by a p73- and caspase 3- dependent process. The goal of the present study was to determine more precisely the pathway involved in the degradation of UHRF1 by TQ.
Material and Methods: Jurkat cells (T lymphoblastic leukaemia cells) and human astrocytoma cells (cell line U87) were used as cancer cell models. Western blot experiments were performed to detect UHRF1, HAUSP and p73 in both cell lines.
Results: We have observed that TQ induced a dose-dependent down-regulation of UHRF1 and HAUSP accompanied with p73 up-regulation. Interestingly, kinetic study revealed the presence of higher bands of UHRF1 expression as assessed by western blotting on both cell lines. Co-immunoprecipitation experiments allowed us to demonstrate, in Jurkat cells, that these bands were due to ubiquitination of UHRF1. These data show that the degradation of UHRF1, challenged by TQ, is due to its ubiquitination through an as yet unknown mechanism but which appears dependent upon HAUSP down-regulation.
Conclusion: In conclusion, we propose that UHRF1 ubiquitination is a key event in the TQ-induced apoptosis in cancer cells. This ubiquitination might result from the auto-ubiquitination activity of UHRF1, following HAUSP down-regulation.

1037 POSTER

The Prolyl-3-hydroxylases (P3H) and P3H-related Genes CRTAP and SC65 Are Novel Transcriptionally Silenced Genes in Burkitt's Lymphoma

C. Lo Nigro[1], N. Syed[2], L. Lattanzio[1], A. Dasoula[3], A. Papoudou-Bai[3], P. Schmid[4], M. Merlano[1], E. Hatzimichael[3], T. Crook[2]. *[1]S. Croce General Hospital, Laboratory of Cancer Genetics and Translational Oncology, Cuneo, Italy; [2]Imperial College London Charing Cross Hospital, Oncology, London, United Kingdom; [3]University Hospital of Ioannina, Hematology, Ioannina, Greece; [4]Brighton and Sussex Medical School University of Sussex, Cancer Medicine, Brighton, United Kingdom*

Background: A number of genes are subject to epigenetic inactivation in Burkitt's lymphoma (BL). Hydroxylation at proline residues is a critical post-translational modification in the biosynthesis of collagen and this mediated by a number of prolyl hydroxylases which act at the 3 and 4 proline positions. Here, we show that the collagen prolyl 3 hydroxylases (P3H) *Leprecan* (P3H1, Lepre), *Leprel1* (Leprecan like1, P3H2) and *Leprel2* (P3H3) and the P3H paralogs, Cartilage Related Protein (*CRTAP; Leprel3*) and Synaptonemal Complex 65 (*SC65; Leprel4*) are targets for epigenetic inactivation in BL.
Material and Methods: We used RT-PCR, immunohistochemistry, methylation specific PCR (MSP) and pyrosequencing to analyse expression and methylation level of the P3H and P3H-like genes in BL cell lines and primary lymphoma biopsies.
Results: Methylation in each of the P3H and P3H-like genes is detected in BL cell lines and primary lymphomas and correlates well with down-regulation of expression. In contrast, the CpG islands are unmethylated or methylated at lower levels in DNA isolated from bone marrow of healthy individuals and in lymphoblastoid cell lines. Of note, there is simultaneous methylation of Leprel1, Leprel2 and SC65 in many BL cell lines and primary BL, implying that the three genes encode proteins with at least partially non-redundant functions. Methylation of Leprel1 and Leprel2 occurs in both sporadic BL and in BL associated with immuno-suppression.
Conclusions: The frequency of transcriptional silencing of the P3H and P3H-related genes in BL, taken together with their known biological properties, implies that prolyl hydroxylation has important functions in lymphoma suppression, loss of which is important in lymphomagenesis.

1038 POSTER

Promoter Hypermethylation of APC, P16, and RASSF1a Genes in Gastrointestinal Cancer

B. Erdem[1], S. Kucukyildirim[1], Z. Polat[2], H. Mergen[1]. *[1]Hacettepe University, Biology, Ankara, [2]Gulhane Military Medical Academy, Gastroenterology, Ankara, Turkey*

Background: Gastrointestinal is the most common cancer type in the world. Cancer is a disease involving dynamic changes of genetics and epigenetics and the formation of tumour is a complex and multi-step process. Abnormalities of DNA promoter hypermethylation which is an epigenetic alteration plays an important role in carcinogenesis. DNA methylation profile is useful marker for tumour diagnosis.
Materials and Methods: 20 tumour tissue samples were collected by endoscopy and/or colonoscopy and 20 blood samples were collected from healty people. After bisulfite modifications of these samples, MSP (Methylation Specific PCR) analysis of *APC*, *p16,* and *RASSF1A* genes were performed.
Results: A relationship between promoter hypermethylation of *APC*, *p16,* and *RASSF1A* genes and tumour progression was found.
Conclusions: Our study suggests that promoter hypermethylation of tumour suppressor genes, such as *APC*, *p16* and *RASSF1A*, is a useful molecular marker for gastrointestinal tumours.

1039 POSTER

Epigenetic Alterations in Lung Cancer Susceptibility Regions

A. Risch[1], D. Scherf[1], N. Sarkisyan[1], W. Hagmann[1], J. Lorenzo Bermejo[2], T. Muley[3], M. Meister[3], H. Dienemann[4], M. Thomas[5], C. Plass[1]. *[1]German Cancer Research Center, Epigenomics and Cancer Risk Factors, Heidelberg, [2]University of Heidelberg, Institute of Biometry and Informatics, Heidelberg, [3]Thoraxklinik/University of Heidelberg, Translational Research Unit, Heidelberg, [4]Thoraxklinik/University of Heidelberg, Department of Surgery, Heidelberg, [5]Thoraxklinik/University of Heidelberg, Department of Oncology, Heidelberg, Germany*

Background: Genome-wide association (GWA) studies have identified lung cancer susceptibility loci, but the corresponding causal mechanisms have not yet been unravelled. Epigenetic mechanisms play an important role in mediating environmental influences on gene expression, and promoter methylation is involved in lung carcinogenesis. Methylation of CpG islands in susceptibility loci was thus investigated as a promising biomarker of lung cancer susceptibility.
Material and Methods: Quantitative high throughput methylation and genotype analyses using MALDI-TOF (Sequenom) were performed. A discovery (n = 34) and a validation (n = 48) set of non-small cell lung cancer (NSCLC) tumours and adjacent normal lung tissue were assessed for methylation in CpG islands located within three lung cancer susceptibility loci. Subsequently, blood samples from lung cancer cases (n = 890) and controls without lung cancer (n = 510), were analysed for *TERT* promoter methylation. A comparative genome-wide methylation analysis after MCIp-enrichment was performed on blood samples from lung cancer cases and controls using whole genome CpG island arrays (Agilent). RT-PCR and *in vitro* expression analyses were also performed.
Results: Tumour hypermethylation was found for the *CHRNA3* and *TERT* promoter CpG islands, while the *CHRNB4* promoter and *TERT* gene body CpG islands were hypomethylated in tumours vs. adjacent normal lung tissue ($p < 001$). Expression data broadly correlated with the observed methylation. Methylation at one *TERT* promoter CpG unit correlated with the genotype at rs421629 ($p = 0.002$). In blood samples of cases compared to controls, the *TERT* amplicon investigated showed a statistically significantly increased average methylation ($p < 0.0001$). In the genome-wide methylation screen, *TERT* was one of the genes identified as differentially methylated between cases and controls.
Conclusions: *CHRNA3*, *CHRNB4* and *TERT* are putative lung cancer susceptibility gene previously identified by GWA studies. This work shows that they are epigenetically dysregulated in lung tumours. The association of tumour-specific *TERT* methylation with *TERT* genotype points to a possible mechanism of the association with lung cancer risk for this locus. The increased methylation found in blood samples from lung cancer cases compared to controls supports *TERT* methylation as an epigenetic marker for early diagnosis. Further investigations are required to determine its value as a susceptibility marker.