The cells were cultured with only GM and GM+C3 inhibitor. Then the number of cells was counted.
Results: The inhibition of HGC proliferation induced by GM was strongly found in time course and a dose dependent manners. In agar electrophoresis(EP), DNA extracts showed a negligible EP pattern. In the TUNEL the cell cultured with GM showed a decrease of total cell number(CN)and increase of positive CN. In IHC staining in P53, ss-DNA, bcl2 and C3 of GM treated cell culture (CC) for 24 hours, total CN decreased and positive cells increased. In the absence of GM, positive cells were scattered. The cell growth inhibition by GM was almost blocked by C3 inhibitor (Table 1).
Conclusions: The EP pattern often is seen in adhesive cells. The purpose of this study was to verify that the cell death is AP, not to investigate the activation rout of AP. To verify that GM causes the AP of cultured cells, it must be confirmed that there is a decrease in the cell count after the addition of GM on CC and that there is inhibition of a decrease in the cell count after the addition of GM+C3 inhibitor on CC. In this study AP was ascertained (Table 1). It was found that GM induced AP not necrosis of HGC. However,as HGC belongs to Type II cell, we speculate the following rout:P53 activation->BH3 activation $\rightarrow$ release of cytochrome c from mitochondria $\rightarrow$ Apf1 activation $\rightarrow$ C3 activation. TUNEL and IHCS showed the staining pattern supported AP. Perhaps, we think that when AP-promoting protein is more predominant than AP-inhibiting protein in the AP rout, AP may be induced. GM will be hoped as a good drug (perhaps to TAE & IV) that attacks to HCC by AP only with a little bit of side effects and without the effect to normal liver.

Table1. The effects of GM+C3 inhibitor on the proliferation of HGC

No.	Culture method	Total cell count
1	HGC for 24 hours culture	26.58×10^5
2	As 1 w/ GM 1,000 μM	18.46×10^5
3	As 2 + C3 inhibitor 80 μM	23.70×10^5 cells/ml

1029 POSTER

P38 MAPK − a Potential Target for Metastatic Melanoma Therapy?

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Background: Metastatic melanoma (MM) is the most malignant of all skin cancers. Only a minority of patients respond to standard therapy, so the perspective for more efficient treatments lies in the development of new strategies for a selective inhibition of growth and elimination of MM cells. Overactivation of the ERK-MAPK signaling is frequently found in MM and is usually caused by mutations of Ras, B-Raf or PTEN. In contrast, the role of the p38 MAPK pathway in MM development and survival is poorly understood.
We have shown previously, that a small molecular inhibitor of p38 α/β MAPK SB202190 (SB) selectively induces autophagy in MM cell lines, but not in other cell types we tested. In our previous experiments, we have demonstrated that SB-induced autophagy promotes the survival of MM cells and combining SB with autophagy inhibitors can lead to a significant decrease of MM cell viability.
Material and Methods: Human melanoma cell lines A375, RVH-421, human osteosarcoma cells U2OS and human foreskin fibroblasts SCRC 1040 were cultured in sterile conditions. Light microscopy of living cells and viability assay with flowcytometry were performed after 24/48-hour of cultivation in the presence of selected inhibitors. Levels of antiapoptotic proteins were analyzed by western blotting.
Results: We investigated the effect of the BH3 mimetic gossypol on MM cell viability as a tool for maximizing the cytotoxic effect of SB and its combinations with autophagy inhibitors. In A375 cells this combination leads to the apoptotic death of nearly 90% of cells. Interestingly, the co-treatment with gossypol is sufficient for efficient induction of apoptosis in SB-treated A375 cells without the need for autophagy inhibitors. In contrast, in RVH-421 cells the addition of gossypol does not enhance the cytotoxicity caused by SB in combination with autophagy inhibitors. Interestingly, SB inhibits expression of Mcl-1 in RVH-421 but not in A375 cells and this might be the reason why further sensitization with gossypol is required for the triggering of cell death in A375 cells.
Conclusions: We showed that SB acts as a cellular stress inducer in MM cells to which they respond by inducing pro-survival autophagy. Our results indicate that increased expression of Mcl-1 protein may be a factor involved in the MM cells resistance to SB treatment and this resistance could be overcome with small molecule inhibitors such as gossypol.
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1030 POSTER

Re-expression of p16 Mediates Apoptosis in Cholangiocarcinoma With Low Rb Level

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Background: Cholangiocarcinoma (CCA) is a malignant bile duct epithelium which is a major liver cancer found in Northeast Thailand. The mortality rate of CCA is high while the survival rate is poor which results from advance stage of the patient at initial diagnosis. The tumour suppressor gene *p16* is a member of the INK4 family of cyclin dependent kinase inhibitor. It functions by direct binding to Cdk4/6 and preventing the phosphorylation of Retinoblastoma (Rb), which in turn blocks cell cycle transition from G1 to S phase. Our previous study showed that loss of p16 protein expression is the most frequent event in CCA (81.5%) and is significantly associated with poor survival. The present study aimed to address the role of re-expression of p16 in CCA cell lines harboring different levels of Rb using adenovirus system.
Materials and Methods: CCA cell lines KKU-100, M055, and M139 established from intrahepatic CCA samples were used in this study. These cell lines expressing no endogenous p16 were infected with p16 recombinant adenovirus vectors (*Ad-p16*) to mediate exogenous expression of p16. The Ad5CMV-Luc vector encoding luciferase was used as a control. Cell cycle and apoptosis were determined by Flow cytometry while beta-galactosidase associated senescence was performed using the X-gal staining method. Subcellular localization and protein levels of p16 and Rb were assayed using immunocytochemistry and Western blotting, respectively. *Rb* knockdown was performed using small interfering RNA (siRNA).
Results: Infection with *Ad-p16* resulted in significantly high level of p16 expression in all CCA cell lines. Exogenous p16 mediated senescence in M055 and M139 cells expressing high level of Rb while KKU-100 which expresses low level of Rb was undergone apoptosis. Apoptosis was observed in *Rb* knockdown M055 and M139 cells infected with *Ad-p16*.
Conclusions: Re-expression of p16 is capable of mediating apoptosis in CCA cell lines through low level of Rb expression and *Ad-p16* may be a promising candidate for cancer gene therapy in CCA.

1031 POSTER

Protein-bound Polysaccharide From Phellinus Linteus Inhibits Tumour Growth, Invasion, and Angiogenesis Through Inhibition of Wnt/β-catenin Signaling in SW480 Human Colon Cancer Cells

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Background: Polysaccharides extracted from the *Phellinus linteus* (PL) mushroom are known to possess anti-tumour effects. However, the molecular mechanisms responsible for the anti-tumour properties of PL remain to be explored. In this study, the anti-cancer effects of PL were examined in SW480 colon cancer cells by evaluating cell proliferation, invasion and matrix metallo-proteinase (MMP) activity.
Background: Polysaccharides extracted from the *Phellinus linteus* (PL) mushroom are known to possess anti-tumour effects. However, the molecular mechanisms responsible for the anti-tumour properties of PL remain to be explored. In this study, the anti-cancer effects of PL were examined in SW480 colon cancer cells by evaluating cell proliferation, invasion and matrix metallo-proteinase (MMP) activity.
Material and metods: The anti-angiogenic effects of PL were examined by assessing human umbilical vein endothelial cell (HUVEC) proliferation and capillary tube formation. The *in vivo* effect of PL was evaluated in an athymic nude mouse SW480 tumour xenograft model.
Results: PL (125–1000 μg/ml) significantly inhibited cell proliferation and decreased β-catenin expression in SW480 cells. Expression of *cyclin D1*, one of the downstream-regulated genes of β-catenin, and T-cell factor/lymphocyte enhancer binding factor (TCF/LEF) transcription activity were also significantly reduced by PL treatment. PL inhibited *in vitro* invasion and motility as well as the activity of MMP-9. In addition, PL treatment inhibited HUVEC proliferation and capillary tube formation. Tumour growth of SW480 cells implanted into nude mice was significantly decreased as a consequence of PL treatment, and tumour tissues from treated animals showed an increase in the apoptotic index and a decrease in β-catenin expression. Moreover, the proliferation index and microvessel density were significantly decreased.

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.
This study was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (R13–2007–020−01000−0), and HanKook Sin Yak Pharm., Korea.

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.
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 (2010–0001290).

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.