200 POSTER

Application of nanobubbles for HSV-tk/GCV cytotoxic gene therapy using ultrasound.

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Herpes simplex virus thymidine kinase (HSV-tk) gene has been demonstrated by several investigators to confirm cytotoxic sensitivity by ganciclovir (GCV) in various tumor cells. Destruction of nanobubbles (NB) mediated ultrasound (US) has been proposed as an innovative method for noninvasive delivering of genes to the tissues of interest. In this report, we evaluated the effectiveness of HSV-tk/GCV cytotoxic gene therapy in cancer cells using NB combined with US *in vitro*.
We transduced HSV-tk gene into five cell lines (A549, MCF7, EMT6, colon 26 and 293T cells) using albmin or lipid nanobubbles (10% v/v) under the optimized US conditions (frequency:945 kHz, duty ratio:50%, pressure: 0.96 MPa). The mRNA of HSV-tk expression was detected by RT-PCR at 24 h after gene transfer. The anti-cancer effects of GCV treatment were evaluated using MTT assay at 6 days after gene transfer. Significant cytotoxity was obtained in only treated cells which expressed the mRNA of HSV-tk, compared to untreated cells.
These results suggest that GCV phosphorylated with HSV-tk would induce specific cytotoxity to transfected cancer cells. We believe that gene delivery using nanobubbles could be useful for cancer specific gene therapy.

201 POSTER

Dolichyl phosphate cycle stimulation as a possible mechanism of mdr1 gene suppression in p-388 leukemia cells

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Background: The investigations reveals that multidrug resistance correlates with MDR1 gene expression and accumulation of P-glycoprotein (Pgp) in plasma membrane. The resent results are in favour of the idea that glycoprotein synthesis in malignant tissues is limited by Dolichyl Phosphate (DolP). Plant Polyprenols (PPol) have been proved to be able to lower tumour cell resistance to chemotherapy *in vivo*. The aim of the present study is to investigate the molecular mechanism of this effect.
Material and methods: P-388 leukemia cells with induced resistance to Doxorubicin (Dox) (P-388/Dox) were obtained by selection from P-388 sensitive leukemia cells (P-388/0) when treating animals with low doses of Dox. Cells were cultivated *in vitro* in RPMI 1640. PPol concentration in the culture medium made up 10^{-3}–10^{-8}M. Pgp extraction from plasma membranes was performed by Riordan and Ling (1979) method. Pgp expression was assessed by an immunohistochemical technique. Dolichyl Phosphate (DolP) and Pgp fractions were analysed by HPLC methods.
Results: Polyprenol in concentration 10^{-3}-10^{-4} M induced apoptosis in leukemia cells within 3–4 hours with nuclear fragmentation and cleavage of genomic DNA. It is confirmed that plasmatic membrans of P-388/Dox cells contain 5.6–6.4% of Pgp (the total protein amount) as a resistance marker. Resistant P-388/Dox cells differ from sensitive ones (P-388/0) in Pgp content by 10–12 times. The study showed 3.5-fold DolP decrease in P-388/Dox cells. The investigations demonstrate that the situation can be changed by resistant cells treatment with polyprenol. The DolP concentration in P-388/Dox cells was returned to the normal level. It is established that DP in the concentration 10^{-6} M aid 7–9-fold reducing P-GP in membranes of P-388/Dox cells. The P-388/Dox cells cultivation in medium with polyprenol proceeded to give lowered Pgp content in membranes no over 0.4–0.6%, which amount was consistent with the level of Pgp in P-388/0 cells.
Conclusions: These results indicate that biosynthesis of Pgp, after MDR1 expression in P-388/Dox cells can be regulated using Dolichyl Phosphate Cycle (DPC) stimulation with polyprenol. Polyprenol isolated from Pinus sylvestris provides a DolP substitute in DPC makes up for a deficiency in the DolP pool. Incorporated into tumour cells polyprenol function as DolP in N-glycosylation of proteins. Polyprenol clinical usage opens up possibilities for pharmacological regulation of drug resistance.

202 POSTER

The role of NF-kappaB in photodynamic therapy-induced apoptosis in lung cancer cells

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Photodynamic therapy (PDT) uses light-absorbing compounds and visible light irradiation to elicit antitumor effects through the generation of reactive oxygen intermediates. NF-κB which is known to be activated from cellular oxidative stress can suppress the cytotoxic effects of radiation and chemotherapy by the transcription of anti-apoptotic genes. On these background, we investigated The role of NF-κB and bcl-2 in PDT-induced apoptosis in lung cancer cells (A549 and NCI-H358).
PDT was performed using Photogem (1, 5, 10, 20 μg/ml) and ALA (10, 100 μg/ml) as photosensitizers and 632 nm laser (1.6, 3.2, 6.4 J/cm^2). Crystal violet assay showed dose- and time-dependent cytotoxicity in PDT-treated cells and apoptosis was confirmed using double staining with Hoechecst 33342 and propium iodide. Luciferase assay showed only weak NF-κB activation by PDT and Western blotting revealed partial IκBα degradation. And also inhibition study using MG132 and triptolode did not show the sensitizing effect of NF-κB blockade. This means that PDT can activate NF-κB weakly and partially in A549 and NCI-H358 cells, but the extent is not enough to affect the cytoxic effect of PDT. In contrast, we demonstrated that PDT-induced apoptosis was inhibited by bcl-2 overexpression.
In conclusion, PDT using photogem and ALA induces apoptotic cell death in lung cancer cells, while it is unlikely that NF-κB plays an important role in PDT-induced cytotoxicity.

203 POSTER

Induction of apoptosis by intracellular potassium ion depletion using the fluorescent dye PBFI-AM in a high-throughput method to follow potassium ion content in cultured lung cancer cells

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Depletion of intracellular potassium ions (K^+) is necessary for cells to shrink, activate caspases and induce DNA fragmentation, events which are features of apoptosis. Here we describe a high throughput method using the cell permeable form of K^+ binding benzofuran isophtalate (PBFI-AM) to measure intracellular K^+ content in relation to untreated control.
Cultured human pulmonary mesothelioma cells (P31) and non-small-cell lung cancer cells (U1690) was treated with K^+ flux modulators in order to deprive the cells of intracellular K^+. The combination of inhibiting K^+ influx (with bumetanide 10 μmol/L and ouabain 10 μmol/L) and simultaneously stimulating K^+ efflux (with amphotericin B 3 mg/L or nigericin 5 μmol/L) was shown to efficiently reduce the intracellular K^+ content after 3h. Manipulation of K^+ fluxes with an ensuing intracellular K^+ depletion was shown to induce apoptosis of lung cancer cells, as an immense TUNEL staining was noted after 3 h K^+ depletion followed by 48 h proliferation.
We conclude that the PBFI-AM assay is a useful tool to measure intracellular K^+ content in relation to untreated control, and that intracellular K^+ depletion of lung cancer cells by clinically used drugs of relevant concentrations induces apoptosis. These findings may lead to novel therapeutic strategies in the treatment of lung cancer.

204 POSTER

Specific interaction between S6K1 and CoA synthase

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Ribosomal protein S6 kinase (S6K) is a key regulator of cell size and growth. It is regulated via phosphoinositide 3-kinases (PI3K) and the mammalian target of rapamycin (mTOR) signaling pathways.
The yeast two-hybrid screen was used to isolate binding partners towards S6K1. One of the interacting molecules was found to encode a novel protein, termed CoA synthase. In our initial studies, we focused on molecular cloning, biochemical and functional characterization of this protein.
We found that CoA synthase mediates the last two steps in CoA biosynthesis via 4'-phosphopantetheine adenylyltransferase and dephospho-CoA kinase activities and termed it CoA synthase. Furthermore, we demonstrated that CoA synthase is localized on the outer mitochondrial membrane and that its activity is strongly activated by phospholipids. Molecular cloning and characterization of CoA synthase provided us with

necessary reagents required to study the specificity of interaction with S6K1 and its functional consequence in mammalian cells.
Here we demonstrate for the first time specific interaction between CoA synthase and S6K1 by co-immunoprecipitation studies in mammalian cells and by BIAcore analysis *in vitro*. The C-terminal regions of CoA synthase and S6Ks mediate the interaction between both proteins. CoA synthase is not a substrate for S6K *in vitro* and its activity is not affected by rapamycin or LY294002 *in vivo*. The physiological relevance of the identified interaction is currently under investigation.

205 POSTER

Nuclear vitamin D receptor regulation of OPN/TCF4/beta-catenin signalling

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Background: Ligand activated nuclear vitamin D receptor (nVDR) may have divergent effects on oncogenesis, through pathways involving β-catenin/T cell factor (TCF4) and osteopontin (OPN) respectively. The nVDR binds to its ligand 1α,25-dihydroxyvitamin D (vitamin D_3), then binds to vitamin D response element (VDRE) in the promoter region to regulate the transcriptions of downstream target genes, e.g. OPN and Wnt signalling including TCF4, TCF1, LEF1 and β-catenin. We wish to test the hypothesis that Vitamin D_3 can either prevent or promote cancer through related mechanisms.
Material and methods: 1) OPN promoter luciferase reporter construct, vitamin D_3 minimum promoter-4XVDRE luciferase reporter construct, TCF-TOP/FOP flash luciferase reporter construct and c-Myc promoter luciferase reporter construct were transiently co-transfected respectively into a rat benign mammary cell line (Rama 37) and Rama 37 invasive cell line (Rama 37-Met-DNA) together with VDR and/or TCF4, β-catenin, LEF1 in their respective expression vectors. Renilla luciferase reporter construct was also co-transfected in each experiment as an internal control. After co-transfection the cells were treated with or without Vitamin D_3 for 48h, then the transactivations of the reporter constructs were detected by assaying the luminescence. 2) Rama 37 cell and Rama 37 cell stably transfected with nVDR were treated with and without Vitamin D_3 or its analogues QW (high activity), BTW (low activity) for 48 h, then the expressions of VDR, OPN, TCF4, TCF1, E-cadherin and β-catenin proteins were analyzed by western blot. 3) Rama 37 cell and Rama 37 cell stably transfected with OPN-pBK-CMV were transiently transfected with VDR, then seeded into the inserts of transwell plate and treated with Vitamin D_3 for 48 h, and the cell invasions were assayed by OD value at 650 nm.
Results: 1) VDR activated by Vitamin D_3 at 10 nM, together with TCF4, β-catenin and LEF1, can transactivate OPN, 4XVDRE and TOP flash promoter luciferase, but not FOP flash and c-Myc promoter luciferase. 2) Vitamin D_3 and its analogues stimulate the protein expressions of VDR, OPN and E-cadherin, but inhibit TCF4 and TCF1 and no significant effect on β-catenin. 3) Vitamin D_3 enhances the invasion capability of transformed cell line, R37-OPN-pBK-CMV, but no effect on benign parental Rama 37 cell.
Conclusions: These results may partly elucidate the regulation loop of OPN/TCF4/β-catenin by VDR in carcinogenesis.

206 POSTER

A new family of KIAA1245 genes with and without the HERV-K LTRs in their introns

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A transcript containing the long terminal repeat (LTR) and the sequence homologous to the KIAA1245 mRNA fragment were revealed among the transcribed LTRs of human endogenous viruses of the K family in normal and tumor tissues. Ten other sequences with a high level of homology to the KIAA1245 mRNA were found in the GenBank. The intron-exon structures were determined for all the sequences, and their exon sequences were compared.
The comparison showed that they differ both in the extent of the exon homology and in the presence or absence of the HERV-K LTR in the second intron. The revealed sequences form a new gene family that comprises at least four subfamilies. Two of these subfamilies have the LTR, and the other two do not. We showed by PCR that the LTR was integrated into the introns after the divergence of the orangutan evolutionary branch from other hominoids but before the divergence of the gorilla branch, i.e., 8–13 million years ago. The total expression of the genes of this family was examined in a number of tissues.
It was shown that LTR-containing genes of this family expressed in tumor, embryonic tissues and in transformed human cell cultures, in explored normal tissues of the mature organism the expression of genes of this family was not detected.

207 POSTER

Transcriptional up-regulation of DNA polymerase by telomerase transcriptional elements-interacting factor

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The over-expression of DNA polymerase β (β-pol) has been identified in lots of human cancers, but the mechanism has seldom been investigated. TEIF (telomerase transcriptional elements-interacting factor) can bind to hTERT promoter, stimulating its transcription and telomerase activities. Here, we report that TEIF could also enhance the expression of β-pol at transcription level.
TEIF could specifically activate transcription of β-pol promoter, but not that of DNA polymerase α or δ promoter. The responsible sequences for binding of TEIF were revealed as GC-rich elements dispersing from +19 to −29 nt of β-pol promoter, which of mutations caused decreasing in binding of TEIF and apparent losing of transactivation activity. The *in vivo* interaction between TEIF and β-pol promoter was identified by chromatin immunoprecipitation (ChIP) assay. Besides, ectopic expression of TEIF in HeLa cells could up-regulate both levels of endogenous β-pol mRNA and protein, and consequently increase resistance to the oxidative stress of H_2O_2.
The data may provide new clue to elucidation of β-pol over-expression in cancers and also a functional link between DNA polymerase β and telomerase.

208 POSTER

Interaction of menadione with a camptothecin analogue in a human colon cancer cell line *in vitro*

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Background: DNA topoisomerase (topo) I and II inhibitors are key drugs of cancer chemotherapy. Irinotecan (CPT-11), a promising camptothecin derivative demonstrating significant antitumor activity to metastatic colorectal cancer, has a unique mechanism of action inhibiting topo I enzyme through the formation of stable topo-I-DNA cleavable complexes. Menadione (MEN), a naphthoquinone compound, is a potent inducer of oxidative stress and apoptotic cell death. Recent studies have shown that it can also activate the *in vitro* disruption of DNA after inhibition of topo II enzyme. At the same time, it has the ability to block cells in G_2/M phase of cell cycle maintaining p34cdc2 kinase in its inactive form. Since MEN is intercepting another path of signal transduction pathway (inhibiting DNA topo II in a different mechanism than CPT-11 inhibits DNA topo I), it is interesting to explore possible interaction with CPT-11. To this end we have assayed the *in vitro* combination effect of MEN with CPT-11 on HT29 human colon cancer cells.
Material and methods: Cells were grown in adherence in 96-well microplates and exposed simultaneously to both agents for 72 hr. Drug cytotoxicity was estimated using the SRB colorimetric assay. The combined drug interaction was assessed with the median-effect analysis and the Combination Index (CI).
Results: CI values illustrated synergistic interaction between the drugs in most concentration ratios applied. The synergy (CI<1) revealed to be slightly greater (CI: 0.135–0.841) for the 1:1 (MEN: CPT-11) concentration ratio, than in 1:2 ratio (CI: 0.520–0.859). For 3.2:1 ratio synergy was abrogated leading to an unequivocal antagonism for all concentration ratios applied and the entire range of cell-kill (CI >1). Our findings indicate that, as a result of synergy, the doses of the tested agents needed to achieve a certain effect (given by Dose Reduction Index-DRI values) may be reduced many times when the agents are given in combination (DRI revealed to be greater than 1 in almost all effect levels). Furthermore, molecular modeling studies for the elucidation of the role of MEN in the docking of CPT-11 in DNA topo I, pointed out the mechanism of the interaction in a molecular level.
Conclusions: The results demonstrate that MEN interacts synergistically with CPT-11. We conclude that CPT-11 may have the advantage of augmenting the anticancer activity in combination with MEN in the treatment of human colon cancer.