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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.

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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.

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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.

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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.

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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