274 Development of folate-lipid-based systems for tumour-targeted gene delivery

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Background: Cationic liposomes have several attractive features for gene transfer and have been routinely used for gene delivery, both in vitro and in vivo. However, their in vivo efficiency is still unsatisfactory, hence it is urgent to optimize their performance by developing novel, efficient and targeted formulations. Cancer therapy requires a selective delivery system to the tumour and therefore lipoplexes can be rendered more selective for cancer cells by targeting them to receptors, such as folate (FA) receptor, which are overexpressed on these cells.
Material and Methods: Lipoplexes were prepared from liposomes composed of DOTAP:Chol and EPOPC:Chol in the presence of FA. FA was associated either electrostatically or attached to liposomes via the PEG spacer arm and the efficiency of these two strategies was compared. The capacity of these systems to deliver reporter genes to TSA (tumour mammary adenocarcinoma) or SCC-VII (oral squamous cell carcinoma) cells in vitro was evaluated, in the presence or absence of serum, by measuring luciferase (LUC) activity and alkaline phosphatase (SEAP) expression. In addition, the antitumoural effect of HSV-Tk/VGCV (valganciclovir) "suicide" gene therapy mediated by FA-associated lipoplexes was investigated.
Results: Our results show that electrostatic association of FA with lipoplexes resulted in a strong potentiation of the biological activity in both cell lines. Importantly, such increase was further improved in the presence of serum and in the case of DOTAP:Chol liposomal formulation. On the other hand, the strategy involving covalent coupling of FA to the liposomes did not result in any significant increase, either in presence or absence of serum. Regarding the application of HSV-Tk/VGCV gene therapy strategy we found that cell death was enhanced with the increase of incubation time and concentration of VGCV.
Conclusions: The electrostatic association of FA to the lipoplexes results in high levels of transfection activity even in presence of serum. The delivery of HSV-tk gene to the cancer cells mediated by FA-lipoplexes and followed by VGCV treatment resulted in a significant therapeutic effect. Overall, these results demonstrate the suitability of the developed systems for the delivery of therapeutically relevant genes in vivo.

275 The effect of Aurora kinase inhibitor, ZM447439, on human mammary eptihelial cell lines with BRCA2 mutation

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Introduction: The Aurora kinases are important participants in mitosis and cell division. Aurora-A and -B amplification or overexpression are common events in various types of tumours. Small molecule Aurora inhibitors have been designed to target tumours with aberrant Aurora expression. Aurora-A amplification has been shown to be common in tumours from BRCA2 mutation carriers and such patients might therefore benefit from treatment with Aurora kinase inhibitors. The aim of this study was to investigate the effect of an Aurora kinase inhibitor on mammary epithelial cell lines with and without BRCA2 mutations.
Materials and Methods: The Aurora kinase inhibitor, ZM447439, was tested on a panel of 14 non-malignant and malignant breast epithelial cell lines that differ with respect to p53 and BRCA2 status. Real-time PCR was used to estimate Aurora-A and -B expression in all cell lines. Cell survival after drug exposure was assessed using crystal violet staining. Cell cycle analysis was performed by flow cytometry.
Results: Treatment with the Aurora kinase inhibitor caused cell death in all the cell lines tested with IC_{50} values ranging from 1.9−8.1 μM. Sensitivity towards the inhibitor did not correlate with levels of Aurora-A and -B mRNA expression, alone. A correlation between high Aurora-A and -B expression was observed. Cells treated with the Aurora kinase inhibitor completed mitosis but cytokinesis was inhibited resulting in polyploidy and multinucleation. Different levels of polyploidy could not be fully explained be defects in p53. Cell lines with a combination of high Aurora-A or -B expression, BRCA2 mutation and p53 defects showed more sensitivity towards Aurora inhibition than other cell lines.
Conclusion: The effect of Aurora kinase inhibitors on survival of breast cancer cells could not be predicted by their level of Aurora-A or -B expression alone. Cell lines with a combination of high Aurora expression, BRCA2 mutation and p53 defects showed the highest sensitivity towards the Aurora kinase inhibitor. Assessment of these three factor could help in the selection of patients who are likely to benefit from treatment with such drugs.

276 Dermaseptin B2: an inhibitor of tumoural growth

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Background: Frog skin secretions contain biological active molecules, like antimicrobial peptides, and have become an important source of inspiration for the discovery of new pharmacological agents, especially useful for drug-based cancer treatments. In this study we investigate if the skin secretions from *Phyllomedusa bicolor* contain such anti tumoural molecules.
Material and Method: Proteins from the skin secretion of *P. bicolor* were separated by a two steps chromatography and tested on the proliferation of tumoural and non tumoural cells, colony formation in soft agar and capillary formation. MALDI-TOF and Automated Edman Degradation were used to identify one of the bioactive compounds contain in the crude extract.
Results: Skin extract showed antitumoural and angiostatic activities and after chromatography procedure one of the bioactive molecules was identified as Dermaseptin B2 (Drs B2). Drs B2 inhibited the growth of different human adherent tumoural cell lines (PC3 and MDA-MB231 carcinomas) with an EC_{50} of 1−2 μM. Drs B2 was likely able to inhibit the lymphoma cell lines Raji and LB-EBV with the same efficiency. It is noteworthy that Drs B2 showed an inhibitory effect on non tumoural cells like NIH-3T3 but only when concentrations higher than 10 μM where used. In addition, Drs B2 inhibits also the endothelial cell proliferation and differentiation *in vitro*.
Conclusion: Drs B2 could represent a new interesting pharmacological molecule against tumoural cell proliferation and the associated angiogenesis. As a perspective, this molecule needs to be evaluated on different cancer cell types *in vitro* and *in vivo*.

277 PPARgamma agonist rosiglitazone enhances the antiproliferative and anti-angiogenic profile of metronomic vinorelbine chemotherapy

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Background: Peroxisome proliferator-activated receptor gamma (PPARγ) agonists have been shown to possess antiangiogenic properties. In the context of our ongoing research interests in metronomic antiangiogenic chemotherapy (Briasoulis *et al*, CCR 2009; Pappas *et al*, EJC 2008) we investigated the antiproliferative and the molecular effects of rosiglitazone (Rg) combined with low-dose vinorelbine (VRL) simulating metronomic administration, on human umbilical vein endothelial cells (HUVEC).
Methods: HUVEC were plated to sub-confluence and were treated for 96 h with different concentrations of VRL and Rg in combination. Medium was replaced every 24 h (metronomic protocol). We assessed the effects of the two agents combined at different concentrations, on cell proliferation and the expression of angiogenesis modulators CD36, CD47, FGFb, IL8, PPARg, TSP-1, VEGF and VEGFr2 at a transcript level (qRT-PCR).
Results: Concentrations of Rg above 10 μM are necessary to induce a significant inhibition of proliferation of endothelial cells when combined with metronomic concentrations of VRL. The half-maximal inhibitory concentrations (IC50) against HUVEC were 10^{-9}M for VRL alone, 10^{-4}M for ROSI alone and 10^{-5}M for Rg when combined with picomolar concentrations of VRL. Combinations of low nanomolar VRL and Rg decreased the mRNA levels of angiogenetic genes IL8, COX-2, CD47 and VEGF while Rg failed to suppress the mRNA levels of these four molecules when cultured endothelial cells were exposed to conventional-dosing concentrations of VRL (10nM). In contrast, combination of VRL with Rg increased the mRNA levels of PPARγ. We did not spot any significant effects on the expression of TSP-1 and CD36.
Conclusion: Our data suggest that PPARγ agonists can enhance the antiangiogenic effects of metronomic vinorelbine.

278 Epigenetic regulation to anti-cancer drugs in HPV positive cell lines

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Chromatin is subject to enzyme effectors that modulates the accessibility of transcription factors to DNA regulatory sequences and hence the gene expression. Some new anticancer drugs are targeting epigenetic factors by their inhibitors in order to reactivate the aberrantly silenced tumour suppressor genes.