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.

The **aim** of this study was to investigate the anti-tumour effect of SAHA (suberoylanilide hydroxamic acid), a histone deacetylase inhibitor, in cell culture models.
Material and Methods: Human papillomaviruses (HPV) immortalized cell lines (CaSki and Hela) and two HPV positive cell lines obtained from cervical xenografts were treated with increasing doses of SAHA (0.25−5 μM). MSPCR and immunotechniques were approached for monitoring certain tumour suppressor genes epigenotype linked with their real time PCR expression analyses.
Results: Flow-citometry analyses revealed that SAHA has an anti-tumour activity by blocking cell proliferation and inducing tumour cell apoptosis in immortalized cell lines. At 2.5 μM/24h SAHA treatment mRNA levels of DNMT1 were slightly increased, as appreciated in real-time PCR (Taqman). In cell lines derived from xenografts DNMT1 activity increases at the same concentration of SAHA after 48 of treatment. In Hela and CaSki lines DNMT3b immunoreactivity presented a rather constant feature, while the only affected enzyme was DNMT3a whose immunoreactivity decreased significantly at high SAHA concentrations (3 μM/48h). The silent versus active state of the considered genes were also estimated by antibody targeting the modified (methylated) histone H3.
Conclusion: HDAC inhibitors may revert the silent heterochromatin to an active chromatin conformation and restore the normal function of silenced genes in cervical cancer. The obtained data suggests any changes in the modifications to either DNA or histone may influence the other.

279 AMPK activators act together with paclitaxel to block tumour growth

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AMP-activated protein kinase (AMPK) and mammalian Target of Rapamycin (mTOR) are key regulators of cellular growth and the aberrant activation of mTOR signaling promotes cell growth, and this underlies the pathophysiology of numerous cancers. Thus, drugs that selectively target AMPK pathway offer great promise for cancer treatment, particularly in combination with chemotherapy. Human tumours were xenografted in SCID mice and treated with low doses of paclitaxel alone, AMPK activators (2-deoxyglucose (2-DG) and metformin) alone, or combination of both drugs. The cellular effects of paclitaxel and AMPK activators were further characterized for breast adenocarcinoma (MCF-7) and lung carcinoma (A549). We observed that treatment with AMPK activators and paclitaxel resulted in an increase in the number of cells arrested in G2/M phase of the cell cycle and decreased tumour growth in mice when compared to individual drugs treatments and control. AMPK activators and paclitaxel alone are able to produce molecular activation of AMPK and inhibition of mTOR signaling in a time and dose dependent manner in MCF-7 and A549 cells. Combined treatment with 2-DG and paclitaxel as well as metformin and paclitaxel lead to quantitative potentialization of molecular signaling through the AMPK pathway by inhibiting mTOR signaling. These findings suggest that AMPK activators interact with paclitaxel in a synergistic manner in lung and breast cancer cells by inhibiting mTOR signaling. Therefore, AMPK activators are a promising therapeutic agent in combination with paclitaxel in lung and breast cancer.
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280 HIV protease inhibitor ritonavir increases heat sensitivity of renal cancer cells by inhibiting heat-induced NF-kappaB activation

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Background: Thermotherapy is one of the treatment modalities against renal cancer, but its efficacy is limited and the ways it affects renal cancer cell survival are not fully understood. In the present study we investigated the molecular mechanism of thermotherapy in renal cancer cells and tried to increase their heat sensitivity by using the HIV protease inhibitor ritonavir, which has recently been shown to inhibit NF-kappaB activity.
Material and Methods: After the heat sensitivity of renal cancer cells (769-P, 786-O, A498, ACHN, Caki-1) had been evaluated by incubating them at 42ºC for 0−60 minutes and assessing cell viability by MTS assay, cells were treated at 42ºC for 0−15 minutes in medium containing 0−50 μM ritonavir before their viability was assessed. Changes in the expression of phosphorylated retinoblastoma protein (Rb); cyclin D1; cyclin-dependent kinase 4 (CDK4); heat shock proteins (HSPs) 27, 70, and 90; NF-kappaB (p65); and phosphorylated p65 were examined by western blot analysis.
Results: In each cell line, treatment at 42ºC inhibited cell proliferation in a time-dependent fashion, especially after more than 15 min, and induced Rb dephosphorylation by suppressing the expression of cyclin D1 and CDK4. The treatment at 42ºC for 15 minutes in the presence of 50 μM ritonavir inhibited cell proliferation synergistically in all the cell lines tested. In Caki-1 cells the treatment at 42ºC for 15 minutes decreased the expression of HSP70, which acts as a suppressor of NF-kappaB, and thus activated NF-kappaB as shown by the increased expression of phosphorylated p65. Interestingly, administration of 50 μM ritonavir in combination with thermotherapy inhibited this increase in phosphorylated p65.
Conclusions: Thermotherapy inhibited renal cancer cell survival by suppressing the expression of cyclin D1 and CDK4. We have for the first time shown that ritonavir increases the heat sensitivity of renal cancer cells, and inhibition of heat-induced NF-kappaB activation is one mechanism of this action. Ritonavir may be used as a heat sensitizer when treating renal cancer by thermotherapy.

281 Cancer: a less depressing outlook? Using antidepressants to induce autophagic programmed cell death in a resistant strain of Burkitt's lymphoma

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Background: Burkitt's lymphoma (BL) accounts for 30−50% of lymphomas in children and 35−50% of HIV-associated non-Hodgkin lymphomas. Survival rates in response to standard chemotherapy are 60% in children but only 25% for older adults and HIV infected patients with reoccurrence and resistance common. Such resistance to chemotherapy is a major obstacle for the success of cancer therapy and is most commonly attributed to the inability of cancer cells to die by apoptosis, the archetypal programmed cell death (PCD) response. The development of anticancer drugs that can overcome this resistance to apoptosis and induce other forms of cell death, such as Type-II autophagic PCD is paramount for efficient cancer therapy and is becoming an increasingly popular alternative therapeutic approach.
Materials and Methods: Apoptotic morphologies in BL cells treated with antidepressants were investigated using Propidium Iodide FACS analysis for apoptotic body detection, agarose gel electrophoresis for the detection of DNA fragmentation, Western Blot analysis for detection of PARP cleavage and the use of the general caspase-inhibitor zVAD-fmk. Type-II autophagic cell death was confirmed by transmission electron microscopy, Western Blot analysis for the detection of the autophagic-specific protein, Beclin-I and the use of the autophagic inhibitors 3-methyladenine and Bafilomycin A1. Mechanisms of cell death were further investigated using confocal microscopy, Western Blot analysis for the detection of Bax and Bak and measuring cytoplasmic calcium levels using FURA-2.
Results: We report that the antidepressants maprotiline and fluoxetine induce autophagic PCD in the chemoresistant Burkitt's lymphoma cell line DG-75, that does not involve caspases, DNA fragmentation or PARP cleavage, but is associated with the development of cytoplasmic vacuoles, all consistent with an autophagic mode of PCD. Autophagic PCD was confirmed by transmission electron microscopy, up-regulation of Beclin-I and the extent of PCD being reduced by the autophagic inhibitor 3-MA. In contrast these compounds, induced apoptotic PCD in the biopsy-like chemo-sensitive BL MUTU-I cell line. We provide evidence that the chemoresistant DG-75 cells do not express the pro-apoptotic Bcl-2 proteins Bax and Bak, show diminished levels of stored intracellular calcium and display shortened rod-like mitochondria, all of which are known to be associated with a defective 'apoptotic' response in cancer cells. PCD in the two cell lines has different Ca^{2+} responses to maprotiline and fluoxetine which may also account for their differential PCD responses.
Conclusions: This study therefore supports a new mechanistic role for maprotiline and fluoxetine as novel pro-autophagic agents in the treatment of resistant Burkitt's lymphoma, and thus an alternative therapeutic application for these compounds.

282 3D-morphometry of squamous epithelium at different stages of malignization

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Background: It is quite common for cytological studies to take into account linear measurements and their ratios for cells in investigation. For example nuclear-cytoplasmic ratio, cell size, size of nucleus and so on. Sometimes 3D-morpfometry is useful as well. 3D-model of tissue based on histological specimens processed by image processing software could be created to improve the differentiation between benign and malignant epithelium specimens. Confocal microscopy is a common tool for 3D-imaging of intracellular structures. In contrast to other works, where the 3D-characteristics of cellular objects were created by computers as a result of 2D-images processing, in present work Atomic Force Microscopy (AFM) has been used for direct measurements of squamous epithelium at different stages of malignization: superficial cels of the cervical squamous epithelium, HPV infected cells (koilocytes), dysplasia, keratinizing squamous cell carcinoma, non-keratinizing squamous cell carcinoma.