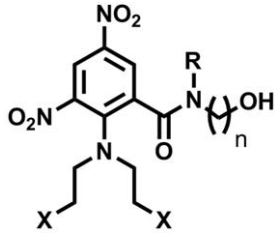
colon, pancreas, lung and glioma tissue. A version of this virus expressing GM-CSF has shown promising results in Phase I and II clinical trials. The present study aims to test OncoVexGALV/CD as an intravesical therapy for superficial bladder cancer.
Material and Methods: In vitro tumour cell killing by OncoVexGALV/CD was assessed by Fusion/Prodrug MTS assays. In vivo efficacy of the treatment was studies through histology and IVIS imaging.
Results: Treatment of three human bladder carcinoma cell lines with the virus, resulted in higher tumour cell killing through oncolysis, pro-drug activation and glycoprotein fusion. To further test OncoVexGALV/CD we have developed a rat orthotopic bladder tumour model to assess intravesical tumour control. Using luciferase-expressing tumours, we will further delineate the effects of individual properties of the virus on tumour growth using the IVIS imaging system.
Conclusions: Preliminary results on human bladder carcinoma cell lines indicate, that OncoVexGALV/CD may improve local tumour control within the bladder, and potentially alter its natural history.

305 POSTER

Structure–activity relationships for lipophilic dinitrobenzamide mustards as prodrugs for Escherichia coli NfsB nitroreductase

S. Sydall[2], A. Ashoorzadeh[1], G.A. Atwell[1], J.B. Smaill[1], W.R. Wilson[2], W.A. Denny[1], A.V. Patterson[2]. *[1]Auckland Cancer Society Research Centre, Medicinal Chemistry, Auckland, New Zealand; [2]Auckland Cancer Society Research Centre, Experimental Oncology, Auckland, New Zealand*

Background: A major weakness of cancer gene therapy protocols is the limited distribution of gene-delivery vectors within the tumour mass. Enzyme-prodrug activating systems (GDEPT) can potentially compensate by generating cytotoxic metabolites that diffuse locally to kill neighbouring vector-naive cells, creating what is known as a 'bystander effect'. E. coli nitroreductase *nfsB* (NTR) in combination with the prodrug CB1954 has been evaluated clinically but efficacy was constrained, at least in part, through inadequate bystander effects (Patterson, Can Res 2002;62:1425). New analogues of the dinitrobenzamide mustard (DNBM) class have demonstrably superior bystander efficiencies in vivo following *nfsB* activation (Singleton, Can Gen Ther 2007;14:953). We sought to maximise metabolite redistribution properties in the DNBM class by modifying the lipophilic nature of the prodrugs through placing additional alkyl groups at three available positions (R, n, X), as well as modification of mustard leaving groups (X).
Methods: A series of 14 novel lipophilic DNBM prodrugs were synthesised and characterised by HPLC, MS, NMR and combustion. Potency (24 h exp) was determined in vitro against $HCT116^{NTR}$ cells (relative to parental cells) in a 5 day proliferation assay. Nine candidates were advanced to mixed 3D tissue culture "bystander efficiency" testing in vitro containing only 1% NTR +ve cells with local toxicity transfer to cocultured WT cells being quantified by clonogenic survival.
Results: Prodrug Log P values spanned >4 orders of magnitude (0.14–4.4). All DNBM prodrugs were more dose-potent (2−60 fold) than CB1954 with $HCT116^{NTR}$ cell growth inhibition (IC_{50}) ranging from 0.05–1.4 μM (28-fold) in a manner that correlated with Log P ($r^2 = -0.82$). Increasing lipophilicity was paralleled with loss of NTR-dependent sensitisation of 2D monolayer cultures. However, only a weak correlation between 2D-IC_{50} and 3D-C_{10} values for $HCT116^{NTR}$ cells was seen ($r^2 = +0.58$), with no apparent relationship with WT-NTR potency ratios in the two systems ($r^2 = +0.24$). As expected, improvements in 3D bystander efficiency (% toxicity transfer from 1% NTR+ve cells) tracked with increasing lipophilicity ($r^2 = +0.69$), ranging from 14% for CB 1954 to 69% for the lead DNBM analogue.
Conclusion: This study establishes the importance of ranking prodrugs using in vitro models with appropriate tissue-like cell densities and identifies several promising leads for further development.



306 POSTER

Targeted suicide gene therapy for small cell lung cancer

C. Laulund Christensen[1], N. Pedersen[1], H. Skovgaard Poulsen[1]. *[1]Department of Radiation Biology, Copenhagen University Hospital, Copenhagen, Denmark*

In suicide gene therapy, the introduced therapeutic gene encodes an enzyme capable of transforming a non-toxic prodrug into a cytotoxic drug. Utilizing cancer-specific promoters suicide gene expression can be selectively targeted to the cancer cells of interest. For that purpose we have identified several promotor regions, which are highly promising candidates for transcriptionally targeted gene therapy for small cell lung cancer (SCLC). The suicide gene yeast cytosine deaminase (YCD) converts the pro-drug 5-fluorocytosin (5-FC) into the known chemotherapeutic agent 5-fluorouracil (5-FU). YCD was cloned for regulated expression from the SCLC specific promoter Insulinoma-associated 1 (INSM1) and transiently transfected into different cell lines, which were exposed to increasing concentrations of 5-FC. Transfected SCLC cells were greatly sensitised to 5-FC and significant cell death was achieved while cancer cell lines of other origins were unaffected to treatment. Furthermore the YCD gene was fused with the yeast uracil posphoribosyltransferase (YUPRT) gene, which augments the conversion of 5-FU into active cytotoxins. The fusion construct (YCD-YUPRT) demonstrated significantly increased sensitivity towards 5-FC in treated SCLC cell lines inducing cytotoxicity comparable to treatment with the 5-FU toxin alone.
Due to limited efficiency of gene delivery in vivo an important feature of suicide gene therapy is the bystander effect where suicide gene/prodrug-produced toxins diffuse to untransfected neighbouring cells. In the cytosine deaminase-based suicide gene therapy 100% cell death was achieved after 5-FC treatment when only 50% of cells expressed the YCD or YUPRT gene. Further it was established that the YCD-YUPRT/5-FC strategy caused extensive cell death when as few as 10% cells expressed the transgene. This contrast previously obtained results with the suicide gene Herpes simplex virus thymidine kinase (HSVtk) and the prodrug penciclovir (PCV) where cell death was restricted to HSVtk-transfected cells.
As succesfull cancer treatment relies on multi-targeting treatment the combination of HSVtk and YCD-YUPRT suicide gene therapy was tested. At low prodrug concentrations an additive effect of the systems was obtained while the YCD-YUPRT mediated toxicity dominated at high 5-FC concentrations. Further testing of these and other suicide systems in vivo will conclude on the significance of combinatorial suicide gene therapy for SCLC.

307 POSTER

Effects of triple knockdown of cIAP-1, c-IAP-2 and XIAP on prostate cancer cell susceptibility to apoptosis

W. Watson[1], C. Gill[1], A. O'Neill[1], C. Dowling[1], J. Fitzpatrick[2]. *[1]Conway Institute of Biomolecular and Biomedical Research, UCD School of Medicine and Medical Science, Dublin, Ireland; [2]Mater Misericordiae University Hospital, UCD School of Medicine and Medical Science, Dublin, Ireland*

Background: Treatments for hormone resistant prostate cancer are currently effective with resistance to apoptotic cell death a common mechanism of resistance in this advanced form of the disease. Manipulation of the apoptotic resistant phenotype represents an important strategy for increasing the response of hormone refractory prostate cancer cells to therapy. Previous studies in our laboratory have identified elevated expression of the inhibitors of apoptosis proteins (IAP) in prostate cancer cell lines and primary material. Knockdown of XIAP is associated with increased susceptibility to chemotherapy induced apoptosis. We hypothesis that simultaneous knockdown of cIAP-1, cIAP-2 and XIAP would further increase the sensitivity of both type 1 and type 2 triggers of apoptosis.
Material and Methods: PC-3 androgen independent prostate cancer cells were treated with optimum concentrations of siRNA to knock down cIAP-1, cIAP-2 and XIAP which was confirmed by western blotting. Following knock down these cells were treated with TRAIL, Etoposide, and Tunicamycin and assessed for apoptosis by PI DNA staining, Annexin V staining and PARP cleavage. Caspase 3 activity was assessed by western blotting and inhibition of apoptosis with the zVAD.fmk pan-caspase inhibitor. Clonogenic assays assessed the ability of the cells to recover following IAP knockdown and ID-1 protein expression was assessed by western blotting as a marker of proliferation.
Results: Triple knock of the IAP only sensitised for TRAIL induced apoptosis in the PC-3 cells with corresponding increases in caspase activity and PARP cleavage which was inhibited by ZVAD.fmk. Individual knock down of the IAP has no significant effects. Triple knock down alone decreases clonogeneic survival of the PC-3 cells which was correlated with a decrease in ID-1 expression.