607 POSTER

Tumour specific delivery of cytostatics encapsulated in novel phospholipase A2 degradable liposomes

S.S. Jensen[1], L.T. Jensen[1], J. Davidsen[1], J.H. Gill[2], S.D. Shnyder[2], M.C. Bibby[2], P.T.L. Høyrup[1], K. Jørgensen[1]. [1]*LiPlasome Pharma, Building 206, Danish Technical University, DK-2800, Lyngby, Denmark;* [2]*Cancer Research Unit, University of Bradford, UK*

Cancer treatment using traditional chemotherapeutics is often problematical due to severe side effects. These side effects could be diminished using specific tumour targeting of the drugs, thereby increasing the drug concentration in the tumour area and lowering the systemic exposure. Liposome based drug delivery has been thought to alleviate these problems, but so far no tumour specific release mechanism has been demonstrated.

We have designed a new generation of liposomes that are specifically degraded by secretory phospholipase A2 type IIA (sPLA2), which is secreted into the tumour microenvironment in a broad range of human tumours. Accumulation of the prodrug liposomes in the tumour is facilitated by the leaky tumour vasculature, known as the enhanced permeability and retention effect.

As a result of the high levels of sPLA2 in the tumour, liposomal phospholipids are cleaved at the *sn-2* position resulting in the release of encapsulated drug and production of free fatty acids and lysolipids, which can act as locally generated permeability enhancers.

We have analysed liposomes loaded with various cytostatics e.g. doxorubicin and cisplatin. The liposomes were evaluated *in vitro* for sPLA2 mediated degradation and concomitant release of the encapsulated compounds. In presence of sPLA2, cisplatin liposomes were degraded with release of cisplatin and lysolipids, acting jointly to cause a strong cytotoxic activity on the cultured cells. In contrast, cisplatin liposomes added to the cells in absence of sPLA2 showed very little cytotoxic activity towards the cells. In contrast, liposomal stealth formulations of cisplatin (known as SPI-077) did not cause cell lysis, in accordance with earlier results.

Xenograft studies with encapsulated doxorubicin and cisplatin in the sPLA2 secreting MT-3 breast cancer model, showed increased therapeutic activity at equimolar doses for both compounds, whereas parameters of toxicity of these formulations indicated similar or weaker toxicity compared to free drug.

These data strongly suggest that sPLA2 triggered tumour specific release of the encapsulated cytostatics is a promising approach for increasing the therapeutic index of current and new cytostatics.

608 POSTER

A new multifunctional drug delivery system based on polymalic acid to inhibit angiogenesis and invasion of human gliomas in vitro and in vivo

J.Y. Ljubimova[1,2], E. Holler[3], M. Fujita[1], N.M. Khazenzon[1], B. Lee[3], K.L. Black[1,2]. [1]*Cedar-Sinai Medical Center, Neurosurgery, Los Angeles, USA;* [2]*Arrogene Inc., Tarzana, USA;* [3]*Institut für Biophysik und Physikalische Biochemie der Universität Regensburg, Naturwissenschaften III, Regensburg, Germany*

Introduction. Specific drug delivery is crucial for treating tumors and reducing side effects for normal cells. Simultaneous inhibition of several molecular targets at the level of protein synthesis may be highly effective in preventing tumor growth and progression. Laminin-8 chains overexpression is associated with glioma progression, and laminin-8 blocking inhibits glioma invasion *in vitro*.

Material and Methods. A multifunctional drug delivery construct consists of modules attached to polymalic acid (PMLA) from *Physarum polycephalum*. The modules are (1) Morpholino antisense oligonucleotides (MOA), which are cleaved in the cytoplasm to release the free drug, (2) antibody to transferrin receptor (ATR) for cancer cell targeting and receptor-mediated endocytosis, (3) short chain PEG-conjugated L-leucine and directly coupled L-valine, to provide pH-dependent lipophilicity to disrupt endosomal membranes, (4) long chain PEG for protection, (5) fluorescent reporter (fluorescein or Cy5) to detect the construct in tissue/cell.

Drug 1: MOA to laminin α 4 and β 1 chain conjugated to PMLA; **Drug 2:** MOA to laminin α 4 and β 1 chain plus ATR conjugated to PMLA. **Controls** were the carrier conjugates with corresponding sense oligonucleotides. Human U-87MG glioblastoma was used for *in vitro* experiments and injected intracranially into NIHRNU-M nude homozygous rats.

Results: The functional effect of module 1 (Morpholinos) was detected as reduced immunostaining for laminin α 4 and β 1 chains; their syntheses were blocked by the antisense oligonucleotides. 2. The functional effect of module 2 (ATR) was detected by fluorescence in cell cultures via fluorescein (PMLA-vehicle) and rhodamine-labeled ATR. They were visible in endosomes and in the cytoplasm equally at different time points. The drug was not toxic in three different concentrations *in vitro* and *in vivo*.

Intracranial tumor treatment. Drug 2 concentrations of 0.5 and 2.5 mg/kg were equal for the treatment in the survival study. After intracranial administration of four doses of Drug 2, the animal survival time was increased by 30%, $p<0.0074$, compared to control groups. Drug 1 (without ATR) did not affect survival. Therefore, the mechanism of drug cell delivery is transferrin receptor-mediated endocytosis.

Conclusions. Antisense oligonucleotides to laminin-8 chains combined with a novel drug delivery vehicle, PMLA, efficiently inhibited laminin-8 expression in a xenografted intracranial human glioma in rats and increased animal survival. The ability of the drug vehicle to penetrate BBB and BTB is important for potential intravenous treatment of patients. These data hold promise for an efficient brain tumor inhibition using laminin-8 as a therapeutic target.

609 POSTER

KCa channel-mediated regulation of metastatic brain tumor permeability and proliferation

N. Ningaraj[4], M. Rao[1], K. Kasisomayajula[1], A. Narasanna[1], R. Thompson[1], D. Hallahan[2], J. Gore[3], T. Yankeelov[4]. [1]*Vanderbilt University, Neurological Surgery, Nashville, USA;* [2]*Vanderbilt University, Radiation Oncology, Nashville, USA;* [3]*Vanderbilt University, Institute of Imaging Science, Nashville, USA;* [4]*Vanderbilt University, Neurological Surgery/Cancer Biology, Nashville, USA*

Background: Brain metastases from breast and lung cancer are a significant source of mortality and morbidity. Since abnormality in receptor kinases (RTKs)/epidermal growth factors is implicated in most cancers, patients with advanced cancer are treated with RTK inhibitors like Trastuzumab (Herceptin), Gefitinib (Iressa), Gleevac and SU11248. A significant number of cancer patients who respond well to these anti-cancer agents, however, develop CNS metastasis. This is most likely due to the inability of anti-cancer agents to cross the blood-brain barrier (BBB) and blood-brain tumor barrier (BTB) to reach cancer cell in the brain in effective quantities. We discovered that calcium-activated potassium (K_{Ca}) channels regulate both BTB permeability and tumor cell proliferation in metastatic brain tumors. The critical role of K_{Ca} channels in tumor cell proliferation provides an opportunity to attenuate tumor cell growth.

Material and Methods: We investigated whether K_{Ca} channels are involved in metastatic brain cancer cell proliferation by FACS, immunohistochemistry and Western blot methods. We also tested whether K_{Ca} channel-mediated BTB opening (with K_{Ca} channel agonists) enhances delivery of RTK inhibitors and enhance survival of rodents with metastatic brain tumors. Animals with metastatic brain xenografts were intravenously administered with [^{14}C]α-aminoisobutyric acid (AIB) and contrast-enhancing agent to develop quantitative autoradiography (QAR) and dynamic contrast-enhanced (DCE) T1-weighted magnetic resonance images, respectively.

Results: K_{Ca} channels are over-expressed in metastatic brain tumor capillary endothelium and brain tumor cells. The prolonged activation of K_{Ca} channels in metastatic brain tumor cells by specific openers, NS-1619 and NS-004 induced K^+ flux, caused membrane hyper polarization, down-regulated K_{Ca} channel expression and activity causing cell death and cell-cycle arrest in the G1 and G2 phases. Normal cells, however, were not significantly affected. BBB/BTB permeability changes measured by DCE-MR imaging before, during and after BTB permeability modulation nicely co-registered with QAR images of rat brain sections. Therefore a novel transitional strategy for high-throughput screening of enhanced anti-cancer drug delivery selectively to metastatic brain tumor was developed.

Conclusion: It is anticipated that these translational experiments will provide a basis for targeting K_{Ca} channel over-expressing metastatic brain cancer cells with pharmacotherapeutic specific openers of K_{Ca} channel openers. Monitoring the outcome of increased RTK inhibitor delivery in patients with metastatic brain tumor should lead to beneficial clinical results.

610 POSTER

Mutant bad effectively inhibited human nsclc xenografts in nude mice

Y. Zou[1], B. Zhou[2], R. Perez-Soler[1]. [1]*Albert Einstein College of Medicine, Medicine, New York, USA;* [2]*M.D. Anderson Cancer Center, Molecular and Cellular Oncology, Houston, USA*

Background: Non-small cell lung cancer (NSCLC) patients frequently present, or relapse, with unresectable disease that is resistant to standard chemotherapy. There is, therefore, an urgent need for new treatments for NSCLC. In order to explore a novel therapeutic method, we have developed orthotopic human NSCLC models and a new therapeutic approach.

Materials and Methods: The human NSCLC models were generated by orthotopically inoculating human NSCLC cell lines H358 or A549 into

nude mice. These xenografts grew multifocally in both lungs and all lobes of the lung and were resistant to intravenous Cisplatin treatment, closely mimicking the NSCLC patients who have unresectable and chemo-resistant tumors. Our therapeutic approach included an efficient nonviral gene delivery system (ENGD) that is composed of multiple cationic polymers at an optimal combination ratio, and a therapeutic gene (*badp*) that is a modified proapoptotic gene, *bad*, that carries mutatant Ser/Thr phosphorylation sites.
Results: *In vitro*, the ENGD-carried mutant *bad* (ENGD-*badp*) significantly induced apoptosis in human NSCLC cell lines H322, H358, H460, and A549. The apoptotic index of cells treated with *badp* was 2- to 6-fold higher than that of the cells treated with wild-type *bad* under the same experimental conditions. *In vivo*, intratracheal injections of ENGD-*badp* effectively inhibited the growth of H358 (%TGI = 61%) and A549 (%TGI = 78%) xenografts in nude mice. In contrast, iv Cisplatin at the maximum tolerated dose was not effective. Moreover, the combination of the ENGD-badp and Cisplatin further increased the average lifespan of the tumor-bearing mice by 60% to 210% compared with the single-agent therapeutics alone (68% vs 7% and 219% vs 7%).
Conclusions: Our studies support the hypothesis that locoregional administration of a proapoptotic gene could effectively inhibit the local chemo-resistant NSCLC tumors and sensitize them for further chemotherapy.

611 POSTER

Introduction of specificity into cytotoxic drugs and improvement of therapeutic index by kinase-mediated trapping

M. Newman[1,2], S. Aspland[1,2], C. Ballatore[1,2], R. Castillo[1,2], J. Desharnais[1,2], T. Eustaquio[1,2], Z. Guo[1,2], Q. Li[1,2], C. Sun[1,2], A. Castellino[1,2]. [1]*Dihedron Corporation, La Jolla, USA;* [2]*Acidophil, LLC, La Jolla, USA*

Non-specific cytotoxic agents continue to play a major role in cancer therapy. In addition to their traditional role, they are essential partners for optimal activity of signal transduction inhibitors in most solid tumor settings and may also be useful in metronomic anti-angiogenic regimens. Despite the continued and potentially expanded use of these agents, their activity is constrained by dose-limiting side effects. Some cytotoxic drugs have been improved via the use of extracellular targeting and pro-drug approaches. However, improvements have been highly drug and disease specific, and suffer from drawbacks with respect to the efficiency of cellular uptake and drug release. We have developed broadly applicable methods for engineering selectivity into non-specific cytotoxic drugs. Our approach takes advantage of well-validated drug discovery targets, i.e. kinases that are aberrantly activated or overexpressed in tumor cells and tumor associated endothelium. Instead of making inhibitors of these cancer-causing enzymes, we have developed methods to covalently conjugate protein and small molecule kinase substrates to cytotoxic drugs. The resulting peptide and small molecule conjugates retain both drug and kinase substrate activities, are stable in serum, and are able to diffuse across cell membranes. We have proposed that selective phosphorylation of the conjugate by an elevated or aberrantly activated kinase can trap the conjugate in the disease or disease-associated cell, preventing exit by passive diffusion and increasing therapeutic index. We have produced bifunctionally active conjugates of paclitaxel and vinblastine with peptide substrates of Src tyrosine kinase and Akt serine/threonine kinase. We have also produced paclitaxel-thymidine conjugates. The conjugates retain 50 to >100% of the parent drug activity and 35 to >100% of the substrate phosphorylation potential. Furthermore, peptide and small molecule conjugates were produced that are stable in serum, exhibit cytotoxic EC50s within 5 to 10-fold of the values obtained for the parent drugs, and in the case of paclitaxel-peptide conjugates, are water soluble. The therapeutic index of each conjugate was determined by comparing cytotoxic EC50s against normal fibroblasts to those obtained with breast, lung and colon carcinoma cells, as well as normal endothelial cells. Conjugates from 4 different drug-substrate classes were obtained that exhibit a 4 to 31-fold increase in therapeutic index, relative to parent drug. Our results demonstrate that it is possible to significantly increase the cell-based therapeutic index of a non-specific cytotoxic agent by linking it to the substrate of a disease-causing kinase. This approach appears to be broadly applicable to non-specific drugs used for the treatment of cancer and many other diseases caused by chronic or undesirable activation of kinases.

612 POSTER

Mitochondrial-mediated apoptosis is induced by cationic polymers used in gene transfer

S.M. Moghimi[1], P. Symonds[2], A.C. Hunter[1], G. Debska[3], A. Szewczyk[3], J.C. Murray[2]. [1]*University of Nottingham, Wolfson Digestive Diseases Centre, Nottingham, UK;* [2]*University of Brighton, School of Pharmacy and Biomolecular Sciences, Brighton, UK;* [3]*Polish Academy of Sciences, Nencki Institute of Experimental Biology, Warsaw, Poland*

A wide range of synthetic polycations in linear, branched, or dendrimer form have been used to condense DNA into structures amenable to cellular internalization via endocytosis. Polycations can destabilize endosomal membranes or act as proton sponges; they buffer the low pH in the endosomes and potentially induce membrane rupture, resulting in the release of polycation/DNA complex into the cytoplasm. The polycationic nature of the gene-delivery vehicles can induce cytotoxicity, but the mechanisms are poorly understood. Therefore, cytotoxic gene-delivery systems may compromise transcription and translation processes and potentially limit protein expression. In order to understand the molecular basis of polycation induced cytotoxicity, we studied the effect of a number of commonly used polycations on mitochondrial functions in isolated mitochondria from rat liver as well as directly in Jurkat cells. Mitochondria are key integrators of a cell's life and death decisions since they play a major role in subcellular partitioning of death-regulating biochemical signals. For example, the Bcl-2-sensitive release of proteins such as cytochrome *c* from the mitochodrial intermembrane space into the cytoplasm is a critical early event in apoptosis. Upon permeabilization or rupture of the outer mitochondrial membrane, cytochrome *c* binds to Apaf-1, leading to allosteric activation of pro-caspase-9. This in turn proteolytically activates caspase-3, one of the principal proteases that participates in the execution of cell death. A decrease in mitochondrial membrane potential ($\Delta\varphi$) due to permeability transition is also an early event in several types of apoptosis. We have demonstrated that at very low concentrations, polycations can affect mitochondrial respiration and $\Delta\varphi$; these events were followed by cytochrome *c* release from mitochondrial intermembrane in mitochondrial suspensions and in Jurkat cells. Changes in mitochondrial $\Delta\varphi$ in Jurkat cells was confirmed by Mitosensor test. Detection of phosphatidylserine translocation to the cell surface using Annexin V, and activated caspase-3 further confirmed the initiation of a mitochondrion-mediated apoptotic programme in Jurkat cells. These observations provide a molecular explanation for the previously reported immediate or delayed cytotoxicity following gene transfer with polycations. The results from this study may help to design novel materials with high transfection efficiencies suitable for clinical gene therapy.

613 POSTER

Plasma and tissue distribution of selenium after 5-methylselenocysteine (MSC) or seleno-L-methionine (SLM) in mice bearing human tumor xenografts

R.G. Azrak[1], L. Pendyala[2], S. Cao[1], F. Durrani[1], J. Prey[2], M. Fakih[2], Y.M. Rustum[1]. [1]*Roswell Park Cancer Institute, Pharmacology and Therapeutics, Buffalo, NY, USA;* [2]*Roswell Park Cancer Institute, Medicine, Buffalo, NY, USA*

Background: We previously reported that MSC and SLM, organic selenium compounds, increase the cure rates of human squamous cell carcinoma of the head and neck xenografts (HNSCC), FaDu and A253, in mice when combined with irinotecan. FaDu xenografts were more responsive to MSC/irinotecan or SLM/irinotecan combination (100% cure rate) than A253 xenografts (60% cure rate). MSC and SLM also protect the animals from irinotecan induced toxicities and lethalities (Cao et al., Clin. Cancer Res., *10*:2561–2569, 2004). To help understand the selectivity of selenium action and its protective effects, we initiated this plasma and tissue distribution study for selenium after MSC and SLM.
Material and Methods: Nude mice bearing bilaterally established (200–250 mg) FaDu and A253 tumors were treated daily with oral MSC at different doses (0.005, 0.01, 0.05, 0.1, 0.2 mg/mouse/d $\times$ 7) or SLM at (0.01, 0.1, and 0.2mg/mouse/d $\times$ 7). Plasma, tumor tissue, and normal tissues (liver, kidney, small intestine, large intestine, and bone marrow) samples were collected at 2h post last dose. Samples were analyzed for selenium concentration using Atomic Absorption Spectrophotometry.
Results: The data show that the base level of total selenium in the plasma of untreated mice is 4.5$\pm$0.5 μM. This level increased to 14.2$\pm$5.1 μM, 23.21$\pm$7.0 μM, and 47.7$\pm$2.1 μM at 2h post SLM administration of 0.01 (the minimal dose for modulation effect), 0.1, and 0.2 mg/mouse/d $\times$ 7 respectively. 94–96% of selenium in plasma is protein bound. The concentration of total plasma selenium increased post administration of MSC (same doses above) to 5.1$\pm$0.56 μM, 9.9$\pm$0.7 μM, and 12.8$\pm$1.6 μM respectively, with 12–21% of total selenium in free form.