121 POSTER

Novel customized releasable polyethylene glycol (PEG) linkers improve tumor delivery and efficacy of locked nucleic acids oligonucleotides

P. Sapra[1], H. Zhao[1], P. Reddy[1], M. Wang[1], R. Bhandaru[1], J. Malaby[1], P. Kraft[1], M. Mehlig[1], L.M. Greenberger[1], I.D. Horak[1]. [1]*Enzon Pharmaceuticals Inc., Research, Piscataway, NJ, USA*

Background: Locked Nucleic Acid (LNA) antisense oligonucleotides (LNA-ONs) represents a new generation of RNA antagonists. Unlike previous chemistry, each LNA monomer contains a methylene bridge between the 2'-oxygen and 4'-carbon of the ribose sugar. This fixes the LNA residue in a favorable RNA-like conformation and enables LNA-ONs to have much higher affinity, specificity, and resistance against degradation compared with other oligonucleotides. While unmodified LNA-ONs have activity in vivo, improved tumor targeting may further enhance efficacy. To address this goal, we have used Customized linker technology to attach polyethylene glycol (PEG) to LNA-ONs via releasable linkers. Different size PEGs were evaluated.
Methods: In vitro efficacy of PEG-LNA-ONs was evaluated in tumor cell lines after transfecting cells with lipofectamine. mRNA knockdown by PEG-LNA-ONs or unpegylated LNA-ONs were evaluated by qRT-PCR. Tumor and plasma distribution of PEG-LNA-ONs were measured in the A549 xenograft model. LNA-ON concentration in organs was evaluated by an ELISA hybridization assay. In vivo, knockdown of target mRNA was evaluated in liver and tumors derived of 15PC3 (prostate) and KB (epidermoid) cells implanted subcutaneously in nude mice.
Results: PEG-LNA-ONs showed good stability in buffers and could be prepared in high yield. In vitro, PEG-LNA-ON resulted in potent knockdown of target mRNA in various cells lines (IC50 < 10 nM). In mice, naked LNA-ONs had a very short circulation time. In contrast, PEG-LNA-ON had >50-fold higher concentration of LNA-ON in circulation (at 2 h and 4 h) compared to naked LNA-ON. At 24 h post injection, PEG-LNA-ON conjugates had 3-fold more accumulation in tumors compared to naked LNA-ONs. The higher molecular weight PEG (40 KDa) conjugates had better tumor accumulation than lower MW PEG (10 KDa) conjugates. Treatment with PEG-LNA-ON (q3dx4) increased knockdown of target mRNA by 2-fold in 15PC3 and KB xenografts models. Additionally, PEG-LNA-ON resulted in >85% knockdown of target mRNA in mouse liver.
Conclusions: Releasable PEGylation of LNA-ONs enhances the tumor targeting and efficacy of LNA-ONs. The improved effects may be due to the enhanced permeability and retention within the tumor, which has previously been observed with PEGylated molecules. Customized PEG linkers may enhance the in vivo delivery of RNA antagonists and subsequently improve efficacy.

122 POSTER

In silico modelling of doxorubicin penetration through multicell layers

R. Phillips[1], P. Loadman[1], P. Jones[2], S. Smye[2], C. Twelves[2], B. Sleeman[2], C. Evans[2]. [1]*University of Bradford, Institute of Cancer Therapeutics, Bradford, United Kingdom;* [2]*University of Leeds, St James Hospital, Bradford, United Kingdom*

Background: Inadequate delivery of chemotherapeutic agents to solid tumours is a significant factor that limits curative potential. The factors which determine drug delivery to tumours are complex but the pharmacokinetic (PK) properties of the drug and its ability to leave the blood vessel and penetrate through avascular tissue are critically important factors. The aim of this study was to develop an in silico model based on these measurements which will predict how far a drug will penetrate from a blood vessel within its PK lifespan. The specific objective of this study is to develop a mathematical model for doxorubicin transport through multicellular layers and to assess the potential impact that efflux via P-Glycoprotein (PgP) may have on drug penetration.
Materials and Methods: Three cell lines were employed; DLD-1 (human colon carcinoma), MCF7 (human breast carcinoma) and MCF7-ADR (Doxorubicin resistant and PgP overexpressing derivative of MCF7). Cells were cultured on Transwell culture inserts to various thicknesses (20–145 μm) as determined by microscope analysis of histological sections. Doxorubicin at various concentrations (100, 50 or 25 μM) was added to the top chamber of the Transwell apparatus and the concentration of drug appearing in the bottom chamber was determined as function of time using HPLC-MS/MS analysis.
Results: In all cell lines, the rate of drug penetration was inversely proportional to the thickness of the multicell layer and the presence of PgP (MCF7-ADR) did not alter the rate of doxorubicin penetration compared to the wild type MCF7 cells. Initial studies have established a mathematical model which is based upon the fact that the transport of doxorubicin across cell membrane bilayers occurs by a passive flip-flop mechanism of the drug between two membrane leaflets. The mathematical model treats the Transwell setup as a series of compartments and the multicell layer is treated as a series of cell layers, separated by small intercellular spaces.
Conclusions: This initial model demonstrates good agreement between predicted and actual drug penetration rates in vitro. Further studies designed to incorporate PK parameters (both real and simulated) into the model are underway with the ultimate objective of making predictions of which schedule of drug administration (bolus vs infusion for example) is likely to be the most efficacious.

123 POSTER

Potent synergy of dual anti-tumor peptides for growth suppression of human glioblastomas using highly efficient peptide-delivery system

E. Kondo[1], T. Yoshino[2]. [1]*Okayama University Graduate School of Medicine Dentistry and Pharmaceutical Sciences, Department of Pathology, Okayama, Japan;* [2]*Okayama University School of Medicine Dentistry and Pharmaceutical Sciences, Department of Pathology, Okayama, Japan*

Background: Molecular targeting agents have become formidable anti-cancer weapons which show much promise against the refractory tumors. Functional peptides are among the more desirable of these nanobio-tools. Intracellular delivery of multiple functional peptides forms a basis for potent, non-invasive mode of delivery, providing distinctive therapeutic advantages.
Materials and Methods: Here we examine growth suppression efficiency of human glioblastomas by dual-peptide targeting. We performed simultaneous introduction of two tumor-suppressor peptides, (p14ARF and p16INK4a, or p16INK4a and p21CIP functional peptides which substitutes for the core function of original genes), as compared with single peptide introduction, using highly efficient peptide/protein transporter (Wr-T)-mediated peptide delivery.
Results: Wr-T-mediated transport of both p14ARF and p16INK4a functional peptides (p14−1C and p16-MIS, respectively) into human glioblastoma cell line, U87deltaEGFR, reversed specific loss of p14 and p16 function, thereby drastically inhibiting tumor growth by >95% within the first 72 hours whereas the growth inhibition was approximately 40% by p14 or p16 single peptide introduction. Additionally, the combination of p16 and p21CIP1 (p21-S154A) peptides dramatically suppressed the growth of glioblastoma line Gli36deltaEGFR – which carries a missense mutation in p53 – by >97% after 120 hours. Significantly, our murine brain tumor model for dual peptide-delivery demonstrated a substantial average survival enhancement (P < 0.0001) for peptide-treated mice. Additonally, we tried our system to the other p14, p16 double negative-cancers, which also showed preferable anti-tumor effect.
Conclusions: Wr-T-mediated dual molecular targeting using anti-tumor peptides is highly effective against growth of aggressive glioblastoma cells, in comparison with single molecule targeting. Thus, jointly restoring multiple tumor suppressor functions by Wr-T-peptide delivery represents a powerful approach, with mechanistic implications for development of efficacious peptide-based molecular-targeting therapeutics against intractable human malignancies which lack tumor suppressor gene functions.

124 POSTER

Nanoparticles as drug delivery device in cancer therapy: investigation of nanodiamond internalization and cellular effects in endothelial and glioblastoma cells

A. Pagano[1], S. Honoré[1], V. Joshi[2], A. Barbier[2], M. Sennour[3], A. Thorel[3], A. Krueger[4], P. Curmi[2], D. Braguer[1]. [1]*INSERM UMR911, Aix-Marseille University, Marseille, France;* [2]*INSERM/UEVE U829, Inserm, Evry, France;* [3]*ARMINES, Armines, Evry, France;* [4]*Christian Albrechts, University of Kiel, Kiel, Germany*

New drug delivery technologies must be designed to surmount biochemical and anatomical barriers and safely allow transport of biomolecules and protein-based drugs to specific intracellular targets. Due to their very low size, nanoparticles have become very attractive. Carbon-based nanomaterials present particular interest, since they are chemically inert, but can be surface functionalized for grafting of nucleic acids, peptides and proteins. This way may allow to specifically target cell compartments and lower drug concentration, reducing side effects. For all these reasons nanodiamond-based therapy may significantly improve cancer treatment.
Aim of the European project "Nano4drugs" was to develop peptide-grafted diamond nanoparticles, including nanodiamonds containing nitrogen-vacancy fluorescent color centers (NV), allowing single particle tracking into the cells. We have studied the uptake and the cellular effects of two kinds of nanodiamonds in endothelial and glioblastoma cells: irradiated fluorescent nanodiamonds (HPF2) and colloidal, functionalised detonation nanodiamonds (OND75). Dose-response (20–100 μg/ml) and